July 2018

Neural and physiological correlates of sex differences in cognition in the marmoset (Callithrix jacchus)

Matthew LaClair
University of Massachusetts Amherst

Neural and physiological correlates of sex differences in cognition in the marmoset (*Callithrix jacchus*)

A Dissertation Presented

By

MATTHEW LACLAIR

Submitted to the Graduate school of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2018

Neuroscience and Behavior Program

Neural and physiological correlates of sex differences in cognition in the marmoset (*Callithrix jacchus*)

A Dissertation Presented

By

MATTHEW LACLAIR

Approved as to style and content by:

_____
Agnès Lacreuse, Chair

_____
Melinda Novak, Member

_____
Jerrold Meyer, Member)

_____
Jacquie Kurland, Member

_____
John M. Lopes, Interim Director
Interdepartmental Programs in the Life Sciences

ABSTRACT

NEURAL AND PHYSIOLOGICAL CORRELATES OF SEX DIFFERENCE IN COGNITION IN THE MARMOSET (*Callithrix jacchus*)

MAY 2018

MATTHEW LACLAIR, BA, EASTERN CONNECTICUT STATE UNIVERSITY

Ph.D. UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Agnès Lacreuse

Sex differences in cognitive performance are well characterized within the literature; however, the neural and physiological correlates of these differences remain elusive. We propose the common marmoset as an ideal model for understanding the neural and physiological underpinnings of sex differences in cognition. The first goal of this dissertation was to examine sex differences in motor ability, stress reactivity, and cognitive ability. The second goal was to examine the ways in which brain metabolite concentration, as measured through Magnetic Resonance Spectroscopy (MRS), and Resting State Functional Connectivity (rsFC) predicted cognitive performance. Motor ability was characterized using the Hill and Valley task, a fine motor task. Both male and female animals showed superior right hand performance when ipsilateral hand and eye coordination was required. When the contralateral hand and eye coordination was required, females outperformed males, potentially indicating superior sensory-motor integration in females. Stress reactivity was measured using a 7-hour social separation paradigm. While overall increases in urinary cortisol did not differ based on sex, females showed a significantly greater rise in cortisol in the first half of the separation.

Additionally, females showed a greater increase in agitated locomotion during separation, indicating greater stress responsivity. Cognition was assessed through Simple Reversal Learning and Intradimensional/Extradimensional (ID/ED) set shifting tasks. Females required more trials to reach learning criterion on the reversal learning trials, indicating poorer performance on this task. No sex differences in ED set shifting were observed. Metabolite concentrations within the prefrontal cortex (PFC) were assessed using ^{1}H MRS. Glx concentration (glutamate + glutamine) in the PFC was correlated with reversal learning performance, and this correlation was significant in males, but not in females. Correlations between resting state networks and reversal learning were investigated using resting state fMRI. Greater network extension of the PFC network was associated with better reversal learning in males, but not in females. Altogether, these findings reinforce the usefulness of the marmoset model of human cognitive performance and indicate that cognition, brain function, and their relationship differ between the sexes.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Sex and gender difference in cognitive performance is a hotly debated topic, both in the public domain and the scientific community. The American Community Survey performed by the U.S. Census Bureau in 2011 found that women are underrepresented in Science, Technology, Engineering, and Math (STEM) fields, with men representing 74% and women 24% of STEM workers. This disparity is particularly apparent in engineering and computer sciences, two areas which make up approximately 80% of all STEM jobs. With the increased focus gender disparities in STEM comes greater interest in the biological and sociocultural factors that impact sex and gender based differences in cognitive performance.

First, it is important to define sex and gender. For the purposes of the current research, sex is defined in terms of gonads and chromosomal makeup, with testes and XY chromosomes indicating male sex and ovaries and XX chromosomes indicating female sex (Einstein, 2017). Conversely, gender is a socio-culturally defined set of characteristics assigned to men and women, related to sex but separate from it. Cultural forces such as stereotype threat, defined as performance-disrupting anxiety induced by fear of reinforcing negative stereotypes associated with one's group identity (e.g. gender) (Steele, Spencer, & Aronson, 2002), have known impacts on cognitive performance. What is less clear is how cultural factors and biological factors differentially contribute to cognitive sex differences.

One way to isolate the effects of sex on cognitive performance, independent of confounding factors, is to use an appropriate animal model of human cognition that

minimizes socio-cultural influences. A nonhuman primate model, the marmoset, is ideal for this research because its cognitive abilities (Spinelli et al., 2004) and brain organization (Belcher et al., 2013) are closely related to those of humans. To control for the contribution of other sexually dimorphic abilities that may modulate the relationship between sex and cognition, motor ability and stress reactivity were also examined. Finally, aspects of brain structure and function that may underlie cognitive sex differences were assessed through MRS (brain biochemistry) and rsFC (resting state functional connectivity). This research is significant because investigating how biological sex impacts cognition is essential for gaining a better understanding of sex-biased cognitive disorders, such as autism, depression, and schizophrenia, as well as sex-specific cognitive dysfunction emerging during reproductive transitions (adolescence, pregnancy and menopause), and in normal and pathological aging.

Cognitive Sex Differences

In humans, there are well-established sex differences in cognitive abilities (Kimura, 1999; Hampson, 2002; Halpern, 2000). Men tend to outperform women on spatial tasks, with the most robust and consistent sex differences found in spatial rotation, in which participants must match a sample object to its rotated pair (Voyer et al., 1995). Men also tend to outperform women on navigational tasks, such as way-finding through 3D virtual mazes (Persson et al., 2013), and remembering the location of targets within a virtual Morris water maze (Astur, Ortiz, & Sutherland, 1998; Chai & Jacobs, 2009; Driscoll, Hamilton, Yeo, Brooks, & Sutherland, 2005; Sandstrom, Kaufman, & A. Huettel, 1998).

Superior male performance on mental rotation has been attributed to the use of more successful "global shape strategy" in which orientation-independent features of the objects are used to determine a match, rather than more time consuming mental rotation of the objects (Hegarty, 2017). The male advantage on navigational tasks has been associated with greater right lateralization of brain activity in the posterior hippocampus (Persson et al., 2013), differences in female and male navigational strategies (Astur, Purton, Zaniewski, Cimadevilla, & Markus, 2016; Sandstrom et al., 1998) and the impact of sex hormones on navigational strategy (Korol & Pisani, 2015 for review).

There are several types of tasks where women tend to outperform men. Women show superior performance to men on tasks of verbal fluency, such as producing words from a specific semantic category (e.g. type of flowers) (Heinzel et al., 2013) and verbal memory, such as recalling words from previously presented lists (Munro et al., 2012; Murre, Janssen, Rouw, & Meeter, 2013) or recognizing words among distractors from a previous list (Murre et al., 2013). The female advantage in verbal fluency may be attributed to an optimal balance of mnemonic clustering (producing words within semantic subcategories) and switching (moving between subcategories) (Weiss et al., 2006).

Most studies also find that women have superior performance on tasks involving memory for location of objects, whether identifying which objects have been moved in a 2-D array or identifying new objects that have been added (Barel, 2016; Duff & Hampson, 2001; Honda & Nihei, 2009; Lejbak, Vrbancic, & Crossley, 2009). However, findings regarding object location are mixed, as some groups fail to find sex differences (Bracco et al., 2011; Epting & Overman, 1998). Voyer and colleagues performed a meta-

analysis which suggests that female advantage is negated when uncommon objects are used in the test array (Voyer, Postma, Brake, & Imperato-McGinley, 2007). Thus, the disparate results in the literature may be due to methodological differences or potentially due to women using an advantageous mnemonic strategy that is impossible when the names of the objects are not known.

Despite this evidence, sex differences may not be as straightforward as previous studies may suggest. Cultural influences, gender stereotypes, and biopsychosocial interactions are known to impact cognitive performance (Miller & Halpern, 2014). Previous work has shown that the magnitude of sex difference on spatial tasks can be affected by socioeconomic status (Levine et al., 2005) and gender equity in a participant's country of origin (Lippa, Collaer, & Peters, 2010). Thus, it may be impossible in human subjects to separate the impact of biological from sociological influences, highlighting the importance of developing appropriate animal models of human cognitive sex differences.

Sex Differences in Motor Ability

Sex differences in motor ability in humans depend on the type of motor task administered. While there is a vast array of tasks used to measure motor function, in general, women outperform men on tasks requiring fine motor coordination (Hall & Kimura, 1995; Jennings, Janowsky, & Orwoll, 1998), whereas men outperform women on tasks involving aiming a thrown projectile (Hall & Kimura, 1995; Watson & Kimura, 1991). There is evidence that the female advantage on fine motor task may be a function of increased estradiol (E2) levels (Jennings et al., 1998; Maki, Rich, & Shayna

Rosenbaum, 2002); however, male performance on fine motor tasks is unaffected by testosterone (T) or E2 levels (Siegel et al., 2008). One group has postulated that greater cross-hemispheric cerebellar connectivity in males is responsible for the male advantage on some motor tasks (Ingalhalikar et al., 2014). Recent work has shown that the strength of rsFC between the left primary somatosensory cortex and the dorsal premotor cortex predicts better motor performance, but how sex impacts these findings is currently unknown (McGregor & Gribble, 2017).

While there is research investigating the hormonal and neuronal underpinnings of motor performance differences, there is a lack of studies exploring how sex differences in motor ability may impact performance on cognitive tasks. While this question may be less germane in humans, it becomes more important in animal models, where differential ability to manipulate objects within a cognitive task has the potential to impact performance. Because we utilize an animal model for this project, it is important to first characterize potential sex differences in motor performance and second, evaluate whether these potential differences modulate sex differences in cognitive performance.

Sex Differences in Stress Reactivity

Sex differences in stress reactivity seem to be dependent on the type of stressor examined. One common stress paradigm used in humans is the Trier Social Stress Test (TSST), in which participants are asked to give a speech and perform mental arithmetic in front of a group of strangers. This task has been shown to reliably increase cortisol and self-reported stress. A recent meta-analysis shows that males show a larger increase in salivary cortisol in response to this task compared to females (Liu et al., 2017). This

increased reactivity to stress in men may be paradigm-specific however, as studies investigating social rejection stress have found women to be more reactive than men (Stroud, Salovey, & Epel, 2002).

There are well established laboratory techniques, like the TSST, for evoking a short-term stress response in humans; however, because of practical and ethical concerns, most studies of the effects of long-term stress are performed using animal models. Chronic stress has known deleterious effects on both neuronal integrity (Lupien & Lepage, 2001; Zaletel, Filipvic, & Puskas, 2016) and cognitive performance (Conrad, 2010) in animals models. In rodent models, evidence suggests a sexually dimorphic response to chronic stress in which male cognitive performance is impaired but female performance is either unaffected or improved (Luine, Gomez, Beck, & Bowman, 2017). This is contrary to findings in humans, showing women to be more prone to anxiety related disorders and more impacted by the negative effects of stress (Bangasser & Valentino, 2014). Acute stress seems to have an inverted U shaped relationship with cognitive performance, in which lower levels of cortisol aid performance and high levels hinder performance (Schwabe, Joëls, Roozendaal, Wolf, & Oitzl, 2012 for review).

While acute stress can have both a positive or negative effect on cognitive performance, what remains to be clarified is how having increased reactivity to stressful events, sometimes referred to as trait anxiety, may impact cognitive performance when individuals are <u>not</u> stressed. Prefrontal cortex (PFC)-related cognitive tasks seem particularly sensitive to the effects of trait anxiety. Previous work has found that increased levels of trait anxiety are associated with decreases in cognitive flexibility and poorer attentional control in mouse models of anxiety (Salomons, Arndt, & Ohl, 2012).

In humans, higher trait anxiety levels were also correlated with decreased cognitive flexibility (Visu-Petra, Miclea, & Visu-Petra, 2013).

For this project, we determined first whether sex differences in stress reactivity exist in marmosets, and second, whether sex differences in stress reactivity have an impact on cognitive performance when animals are not simultaneously being stressed.

Neuroimaging Tools: Differences in Brain Metabolites

Metabolite concentrations in the brain can be measured non-invasively using *in vivo* magnetic resonance spectroscopy (MRS). This brain imaging technique uses signal from hydrogen molecules to determine the concentration of several metabolites including N-acetyl aspartate (NAA), myo-Inositol containing compounds (mI), Choline containing compounds (Cho), Glutamate (Glu), Glutamine (Gln), and Phosphocreatine+Creatine (Cr) (Gujar, Maheshwari, Bjorkman-Burtscher, & Sundgren, 2005). MRS has shown to be a useful tool in investigating potential biomarkers of cognitive performance in the healthy human brain (Patel, Blyth, Griffiths, Kelly, & Talcott, 2014; Ross & Sachdev, 2004).

Several metabolites show positive correlations with cognitive performance. Levels NAA are used as a marker for neuronal health and integrity in cortical tissues (Jung et al., 2005). NAA level within the left frontal and left occipito-parietal brain regions positively correlates with selective aspects of cognitive performance in healthy populations, and this relationship has been found to be stronger in women than in men. However, the sex differences in strength of the association between NAA and cognitive performance maybe region-specific, as another study found a positive correlation

between NAA and cognitive performance in right posterior grey matter and a negative correlation within right anterior grey matter, independent of sex (Jung et al., 2009).

Glutamate is the most abundant excitatory neurotransmitter in the brain and is known to play a role in learning and memory. While studies have not found a sex difference in Glx (Chang, Jiang, & Ernst, 2009), a combination of glutamate and glutamine concentrations, it has been shown to predict verbal memory performance in healthy adult males (Wagner et al., 2016) and in older adults (Nikolova, Stark, & Stark, 2017), with increased concentrations in the hippocampus associated with superior verbal memory. Unfortunately, these previous studies did not include sex as a variable, so sex differences in the impact of Glx concentration on verbal memory were not assessed.

Neuroimaging tools: Differences in Resting State Functional Connectivity

rsFC is a recent fMRI technique that examines temporally correlated fluctuations in the blood oxygen level dependent (BOLD) signal when participants are at rest (not completing a study-related task). Since Biswal and colleague's landmark study in 1995, which showed that low frequency fluctuations in activation within the sensorimotor cortex when participants were at rest were correlated with patterns of task-based activation, there has been increasing interest in understanding how fluctuations in brain activity while at rest may reflect structural connectivity.

Variations in resting state signal have been shown to also have an impact on cognitive function in healthy participants. For example, the strength of rsFC signals has been shown to be positively correlated with working memory performance (Sala-Llonch et al., 2012; Zou et al., 2013) Mounting evidence also suggests that sex impacts the

strength of resting state networks (Allen et al., 2011; Filippi et al., 2013; Tian, Wang, Yan, & He, 2011). It is logical to assume that these differences in rsFC are likely contributing to cognitive sex differences; however, there is a paucity of data regarding how sex differences in rsFC networks may relate to sex differences in cognitive performance.

The overarching goal of my experiments was to examine sex differences in cognition using a small primate, the common marmoset (*Callithrix jacchus*) as a model system. I will first discuss the more traditional primate model of human cognition, the macaque monkey model, before highlighting some of the advantages of the marmoset for cognitive sex difference research.

The Rhesus Monkey Model of Cognition

Non-human primates (NHPs) are particularly useful models of human cognition for several reasons. Compared to other animal models, NHPs are generally more similar to humans in terms of cognition (Camus, Ko, Pioli, & Bezard, 2015), physiology (Sereno & Tootell, 2005), and brain organization (Preuss, 2012; Rilling, 2014). Macaque monkeys (*Macaca mulatta and Macaca fasicularis)* are particularly useful primate models in neuroscience research, given their close phylogenetic relation to humans: the last common ancestor for macaques and humans existed approximately 25 million years ago. Macaques' ability to quickly learn and perform complex cognitive paradigms have made them an important model in studies examining cognition and cognitive aging

(Lacreuse & Herndon, 2009). Unfortunately, relatively few studies in macaques have examined the impact of sex on cognitive performance.

While the data on sex differences in cognitive performance in macaques are sparse, results suggest that sex difference in performance exist. Lacreuse et al. found a male advantage for young adult rhesus monkeys in spatial working memory, in agreement with the human literature (Lacreuse et al., 2005). However, Herman and Wallen found a female advantage on a navigational task that traditionally show a male advantage (Herman & Wallen, 2007), thus results are inconsistent regarding male/female advantage in spatial ability.

While there are clear advantages to the macaque model, there are also drawbacks to utilizing this species of monkey. Because macaques can carry deadly disease pathogens, additional training and precautions are required when working with these animals. While the projects by Lacreuse et al. and Herman and Wallen included a large number of subjects ($n = 90$ and $n = 51$, respectively), the cost and space required for housing rhesus monkeys makes studies of this size unique. Because the cost and space of housing larger primates can sometimes lead to small sample sizes, sex is not always feasible to assess in cognitive studies using macaque monkeys. Alternative models are necessary to achieve sample sizes required to assess sex differences. My project examined the validity of the common marmoset as such a model.

The Marmoset Model of Cognition

The marmoset has recently been proposed as an alternative model for human aging (Tardif et al., 2011) and has many characteristics that distinguish it from the rhesus monkey and make it ideal for this type of research. Marmosets are considered old at 8 years of age (Abbott et al., 2003), have large brains relative to their body size (300-500g), exhibit functional brain networks similar to humans (Belcher et al. 2013), and are capable of performing complex cognitive tasks (Spinelli et al., 2004).

Marmosets are small and relatively short-lived. At around 300-500g, marmoset body weight is approximately 4% of an adult rhesus monkey's weight, allowing researchers to handle marmosets with relative ease. Because of their small size, marmosets have lower feeding and caging requirements than traditional lab primates, which translate to lower overall cost and allows for experiments with larger sample sizes. Additionally, marmosets are one of the shortest-lived anthropoid primates, with a lifespan of approximately 10 years (Nishijima et al., 2012). Compared to macaques, with an average life span of 40 years, longitudinal studies are much more feasible in marmosets.

Functional connectivity within the somatosensory cortices and large-scale brain networks has recently been studied in awake marmosets (Liu et al., 2013). Marmosets show four higher-order functional connectivity networks that are similar those found in humans, including a default mode-like network, an orbitofrontal network, a frontopolar network, and a salience-like network (Belcher et al., 2013). However, the effects of sex on functional connectivity have yet to be elucidated in the marmoset. Validating the use of a marmoset model, which can be imaged with ultra-high field MRI, would be

extremely useful for detailed connectivity mapping and understanding how functional connectivity is related to cognitive performance.

Although marmosets are more evolutionarily distant from humans than rhesus monkeys (35 M), marmosets are able to perform many of the cognitive tasks used in rhesus models of human cognition. Spinelli and colleagues validated the marmoset model of human cognition using the CAmbridge Neuropsychological Test Automated Battery (CANTAB), a computerized touch screen cognitive testing battery used in both humans and rhesus monkeys (Spinelli et al., 2004). Marmosets had stable performance that was comparable to rhesus monkeys, on three CANTAB tasks: the progressive ratio task, measuring motivation for reinforcer; the five-choice serial reaction time task, measuring attention; and the delayed matching to position task, measuring working memory.

The endocrinology of the marmoset shows several differences with that of Old World monkeys and humans. Marmosets have a 28-day menstrual cycle, but have much higher levels of sex steroids (Dixson, 2012). They lack external signs of menstruation and show a shorter follicular phase (around 8 days) and a longer luteal phase of around 20 days. Along with tamarins, marmosets are the only anthropoid primates to exhibit multiovulation (2-4 eggs ovulated) and they usually give birth to twins or triplets (Tardif & Bales, 2004).

Overview, Hypotheses, and Predictions

Subjects and General Procedures

Our colony consists of 28 marmosets ranging from 4 to 6 years old. Because not all animals participated in all paradigms, number of animals used and mean age for males and females are indicated within the description of each experiment. Characteristics of the marmosets can be seen in **Table 1.1**.

All marmosets were housed in male/female pairs at the University of Massachusetts, Amherst and maintained under a 12:12 light/dark cycle (lights on at 7:30AM) at an ambient temperature of 80 F with a relative humidity of 50%. The pairs were housed in steel mesh cages (101 x 76.2 x 78.7 cm) equipped with perches, hammock, nest boxes and branches to encourage species typical behaviors. Male marmosets were vasectomized in adulthood, prior to the start of the study, to avoid pregnancy. All animals were trained using positive reinforcement to enter transport boxes openings attached to the front of their home cages. Transport boxes (34.1 x 20.65 x 30.8 cm) were constructed of Plexiglas on three sides and a metal meshed front with 2.5 x 2.5 cm, which allowed animals to reach through and manipulate study materials. Once trained to enter the transport box voluntarily, most experimental procedures occurred while the animal was within the transport box.

Animal ID	Sex	DOB	Age at Start of Cognitive Testing	Age At Social Separation	Age at Motor Testing	Age at MRS Imaging	Age at rsFC Imaging
02	Female	9/16/10	5.82	5.52	5.49	4.40	6.52
04	Female	9/16/10	5.32	5.59	5.41	5.42	6.56
06	Female	7/5/11	4.93	4.67	4.68	4.79	5.64
08	Female	1/4/10	6.05	6.16	6.09	N/A	7.32
10	Female	7/5/11	4.52	4.81	N/A	N/A	5.93
12	Female	3/22/11	4.82	5.01	4.95	N/A	5.76
14	Female	11/23/11	4.12	4.36	4.26	6.21	N/A
15	Female	1/18/11	4.99	5.21	5.14	N/A	6.45
17	Female	4/2/12	4.21	4.01	4.07	5.77	5.53
19	Female	1/6/12	4.78	4.25	4.35	N/A	N/A
21	Female	11/18/11	4.72	4.71	4.47	5.02	5.61
23	Female	4/28/12	4.08	3.96	3.96	3.93	5.13
26	Female	3/18/12	4.41	4.34	4.44	5.52	5.70
28	Female	3/28/12	4.55	4.34	N/A	N/A	N/A
01	Male	6/1/11	4.64	4.87	4.78	4.98	5.82
03	Male	6/18/10	5.56	5.73	5.72	4.34	6.81
05	Male	5/1/11	4.69	4.86	4.84	4.72	5.81
07	Male	9/3/09	6.86	6.51	6.48	4.88	7.65
09	Male	8/20/10	5.52	5.57	5.52	4.83	6.80
11	Male	10/28/10	5.21	5.39	5.27	4.32	6.15
13	Male	11/28/11	4.65	4.64	N/A	N/A	N/A
16	Male	5/13/12	3.96	3.92	N/A	N/A	5.13
18	Male	5/10/11	5.30	4.93	5.01	N/A	6.42
20	Male	4/8/11	5.21	5.04	5.05	5.19	N/A
22	Male	6/4/11	5.01	4.88	4.93	N/A	6.07
24	Male	11/9/11	4.77	4.41	4.42	N/A	5.59
25	Male	8/4/11	N/A	5.06	N/A	N/A	N/A
27	Male	9/28/11	5.03	4.81	4.92	N/A	6.17

Table 1.1 Characteristics of study subjects: Sex, Date of Birth, and Age at Test. N/A indicates animal did not complete test

The marmosets were fed Teklad New World Primate Diet (Envigo, Madison, WI) supplemented with Zupreem marmoset diet, and a variety of fresh fruits, vegetables, nuts, and mealworms, up until 2 hours before and immediately after cognitive testing. The monkeys were provided with daily enrichment. The animals were cared for in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals, 8th edition. The studies were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts at Amherst.

Sex differences in behavioral outcomes: Motor, Stress Reactivity, and Cognition (Chapter 2)

Experiment 1 examined whether sex and/or hand preference affected performance on a task of fine motor control, using the Hill and Valley task. The Hill and Valley task is used in stroke and Parkinson's research in the marmoset, and measures motor function in each limb as well as visual-spatial impairment. To our knowledge, the impact of sex on Hill and Valley performance has not been examined, however, based on research on fine motor control in humans, we anticipated a female advantage on this task, with the prediction that females would perform faster and make fewer errors than males on both the Hill and Valley tests.

In Experiment 2 we investigated sex differences in behavioral and endocrine responses to social separation, in which the focal animal was removed from the colony and placed in isolation for 7 hours. During separation, urine was collected hourly to assess changes in urinary cortisol, and behavior was recorded on videotape for later analysis Based on previous research in our lab, we expected greater stress reactivity in

females, reflected by a more robust behavioral and endocrine response to the social stressor. We predicted that females would have (1) a greater increase in cortisol during the separation than males, and (2) would engage in more behavioral indications of distress (e.g. alarm calls, agitated locomotion) than males.

In Experiment 3 we used two cognitive tasks to examine the effect of sex on cognitive performance. Tasks were administered using the CANTAB. Marmosets completed the Reversals test, in which animals must choose one of two target stimuli to receive reinforcement (simple discrimination) and then adjust their performance when the reward contingencies are reversed (reversal), and the intradimensional/extradimensional set shifting (ID/ED) task, in which animals choose the correct stimulus (i.e. one of two shapes) while ignoring an overlaid extraneous stimulus (i.e. two lines).

It is currently unknown if marmosets show sex differences in cognitive function, so hypotheses on sex differences in performance could not be based on literature in marmosets. One recent report failed to find sex differences in a group of 35 young marmosets (1-4 years old) performing visual discriminations and reversal tasks (Takemoto et al., 2015). However, it is unclear if this pattern of performance is maintained in older animals and in more complex cognitive tasks. Based on my preliminary studies and human literature in which analogous tasks are utilized, we hypothesized a female impairment in reversals and ID/ED, reflected by an increase in both trials and errors to learning criterion. We predicted that females would take more trials and make more errors, to reach learning criterion than males on both the Reversal task and the ID/ED.

Sex differences in neuroimaging measures: Magnetic Resonance Spectroscopy (MRS) and Resting State Functional Connectivity (rsFC) (Chapter 3)

Experiment 4 examined sex differences in the concentration of neurometabolic markers of cognitive performance in the PFC, and whether these metabolites were correlated with cognitive performance on the Reversals task. We measured concentrations of N-Acetyl Asparate, Myo-Inositol containing compounds, Choline containing compounds, Phosphocreatine + creatine, Glutamate (Glu) and Glutamine (Gln) using a 3 mm^3 voxel positioned in the PFC. Based on previous research showing that Glu receptor blockade impairs reversal performance in marmosets (Harder, Aboobaker, Hodgetts, & Ridley, 1998) we expected that Glu or Gln or Glx (Glu + Gln) concentrations would be correlated with performance on the Reversals task. It was predicted that an index of reversal performance, the reversal index, (computed by dividing mean trials to criterion on reversals by mean trials to criterion on initial discriminations, with lower values indicating better performance) would be negatively correlated with Glu, Gln and Glx concentrations.

The main objectives of Experiment 5 were to assess sex differences in rsFC, and examine whether sex differences in rsFC correlated with cognitive performance on Reversal Learning. Animals were trained to undergo awake neuroimaging and scans were analyzed using independent component analysis to identify 25 resting state networks. Because data on sex differences in rsFC in marmosets is sparse, we did not have specific hypotheses in this regard, however, we did hypothesize that increased connectivity within the PFC when at rest would be associated with better performance on

the reversals. Specifically, we predicted increased connectivity within the PFC to be negatively correlated with the reversal index.

Brain/Behavior relationships and General Discussion (Chapter 4)

The main objective of this set of analyses was to examine whether sex differences in motor function, stress reactivity, metabolite concentration in the PFC, and rsFC predicted cognitive performance. We performed a multiple regression analysis to assess the contributions of each of these factors to the variance in cognitive performance. This analysis is followed by a general discussion of the overall research findings.

CHAPTER 2

SEX DIFFERENCES IN BEHAVIORAL OUTCOMES: MOTOR, STRESS, AND COGNITION

Experiment 1: Sex Differences in Motor Control

In humans, males and females each have an advantage on different types of motor tasks. Generally, men tend to outperform women on "targeting" tasks, which involve throwing an object (such as a ball or a dart) at a predetermined target (Hall & Kimura, 1995), or using hands to intercept a projectile object (Watson & Kimura, 1991). These differences are generally found to be independent of differences in participant experience with sports that involve targeting (Hall & Kimura, 1995; Watson & Kimura, 1991) or sex differences in size and strength (Watson & Kimura 1991).

While men have an advantage in targeting tasks, women seem to have an advantage on fine motor tasks, such as the Purdue Pegboard Task, in which participants must pick up pegs and place them in appropriately sized holes as quickly as possible (Hall & Kimura, 1995) (Nicholson & Kimura, 1996) and the sequential movement task, in which participants are taught to press buttons in a predetermined order (Jennings et al., 1998). This female advantage on fine motor tasks could be due to differences in sex hormones, as women have faster performance on fine motor tasks during the midluteal phase of the menstrual cycle, when estradiol levels are high, than the follicular phase, when E2 levels are lower (Jennings et al., 1998; Maki et al., 2002), although this association between E2 level and sequential movement is not found in all studies (Hampson & Kimura, 1988). While there is a connection between sex hormones and fine motor control in females, neither testosterone or E2 levels seem to be associated with

male performance on fine motor tasks (Siegel et al., 2008).

Data examining the effects of sex on motor ability is sparse in NHPs. Lacreuse and colleagues (2005) found that old male rhesus monkeys performed worse than young males and females of any age on a fine motor task that required animals to retrieve Lifesaver candies from rods of increasingly complex shapes, indicating a greater decline in performance in males but no sex differences in performance in younger adult animals.

The marmoset is quickly becoming a popular model for understanding the effects of motor disease and injury because of the similarities between the marmoset cortical motor system and those of higher primates. The marmoset frontal cortex contains anatomically discrete motor areas, similar to the organization of this region in other primate species (Burman, Bakola, Richardson, Reser, & Rosa, 2014). This region, the marmoset primary motor cortex (M1) is organized like other primates: it is defined by a single functional field, containing a topographical map of the body musculature (Burish, Stepniewska, & Kaas, 2008). These organizational similarities make marmosets a suitable model for human motor function.

To our knowledge, no study has investigated the effect of sex on motor ability in marmosets, however, determining whether sex affects motor proficiency in marmosets is important in establishing an appropriate model for human motor disease and injury. To assess motor function, we used the Hill and Valley task, a measure of fine motor ability that has previously been used in marmosets, especially in models of stroke and Parkinson's disease (Bihel et al., 2010; Eslamboli, Baker, Ridley, & Annett, 2003; Le Gal, Bernaudin, Toutain, & Touzani, 2017; Marshall et al., 2003; Phillips et al., 2017). It assesses motor function in each limb as well as potential perceptual spatial impairment.

Subjects

Twenty-one monkeys, 11 females (mean age = 4.78 years, SD = 0.69) and 10 males (mean age = 5.05 years, SD = 0.40), completed the Hill and Valley task.

Methods

The monkeys were tested in their housing room. They voluntarily entered a transport box (34.1 x 20.65 x 30.8 cm) attached to their home cage to access the Hill or Valley apparatus, securely attached to the front of the box via a Plexiglas screen. Each apparatus had two 5-steps (9 x 9 x 3 mm) staircases, either rising away from a central opening (Valley), or from two lateral openings (Hill) (see Fig. 2.1 for images of apparatuses). The monkeys had to reach through these openings, using either their right or left hand, to retrieve one of the mini dehydrated marshmallows (6 mm diameter) placed in the middle of each step. In the Valley version, the central vertical slot (7.7 x 2 cm) allowed the marmoset to use its left hand to reach the reward located on its right, or the right hand to reach the reward located on its left (contralateral hemifield to hand used). In the Hill version, entry was through two lateral slots (7.4 x 2 cm) on the side of each stair so that the monkey had to use its right hand to retrieve the rewards on the right stairs and the left hand to retrieve the rewards on the left stairs (ipsilateral hemifield to hand used).

Marmosets were trained on the Hill and Valley apparatus until they successfully retrieved a dried mini marshmallow from each step with each hand. If the marmoset failed to perform the task after a maximum of 10 training attempts, it was excluded from the task. For testing, marmosets were given a maximum of 5 min to retrieve all 5

marshmallows from one staircase of the apparatus. Each marmoset received 4 conditions (Hill Left, Hill Right, Valley Left, Valley Right) per session, one session per day, and performed a total of 3 testing sessions. The order of the Hill and Valley conditions was randomized (half received Hill first, half Valley first) and alternated each test day. If the marmoset failed to retrieve the 5 marshmallows within the 5-min time limit, the test session was rerun the following day. Marmosets received 1 point for retrieving the marshmallow on the 1st step, 2 points for retrieving from the 2nd step, and so on, for a maximal score of 15 points per hand. Marmosets lost 1 point each time a marshmallow was dropped. The latency from the first reaching through the opening until retrieval of the last marshmallow was recorded for each condition.

Hand preference

Because hand preference had the potential to affect hand performance, we first determined the hand preference of each marmoset using a simple hand reaching task. Monkeys performed 50 reaches through the central slot of the Valley apparatus to reach a mini marshmallow placed 1 cm from the slot. The number of Left and Right hand reaches was recorded. Any trials in which the marmoset used both hands were excluded. For each subject, a handedness index (HI) was determined by subtracting the number of left-handed responses from the number of right-handed responses and dividing by the total number of responses. HI values ranged from −1.0 to 1.0, with the absolute value representing the strength of the preference. The positive values indicated a right-hand bias while the negative values indicated a left-hand bias. In addition, subjects were classified as left-, ambidextrous, or right-handed based on binomial z scores calculated from the frequency of left- and right-hand responses. Subjects with z scores of −1.64 or

lower were classified as left-handed and those with z scores of 1.64 or higher were classified as right-handed. All others were classified as ambidextrous.

Animal ID	Sex	Handedness Index	Z-Score	Hand Preference
03	Female	-0.40	-2.83	Left
08	Female	-0.56	-3.96	Left
12	Female	-0.48	-3.39	Left
14	Female	-0.72	-5.09	Left
19	Female	0.12	0.85	Ambidextrous
20	Female	0.28	1.98	Right
21	Female	0.48	3.39	Right
23	Female	0.72	5.09	Right
24	Female	-0.28	-1.98	Left
26	Female	0.76	5.37	Right
28	Female	0.48	3.39	Right
01	Male	-0.08	-0.57	Ambidextrous
02	Male	0.48	3.39	Right
04	Male	-0.08	-0.57	Ambidextrous
07	Male	-0.32	-2.26	Left
09	Male	0.08	0.57	Ambidextrous
10	Male	0.92	6.51	Right
13	Male	0.12	0.85	Ambidextrous
15	Male	-0.32	-2.26	Left
17	Male	0.20	1.41	Ambidextrous
25	Male	0.48	3.39	Right

Table 2.1 Handedness index, Handedness Z-score, and hand preferences for the 21 marmosets that completed the Hill and Valley task

Statistical analysis

Analyses were performed on each test (Hill or Valley) separately. Mixed measures ANOVAs were performed on the latencies and scores, with Sex, Hand used, and Hand Preference as factors. As Hand Preference was not significant and did not

interact with any other factors for any of the two variables, it was removed from subsequent analyses.

Results

Based on a simple reaching task, 7 monkeys were classified as left-handers, 9 as right-handers and 5 as ambidextrous (see Table 2.1). There were no sex differences in the number of animals classified as right handed, left handed, or ambidextrous ($\chi^2(4) = 2.35$, $p = .67$) The strength of the lateral bias (HI) did not differ between left and right handed individuals (independent t test, $t(14) = -1.01$, ns). We examined the effects of Sex, Hand Use on the latencies to complete the tests as well as scores, for each test separately.

For the Valley test, Hand Use had a significant effect on the scores, with the right hand obtaining better scores than the left hand ($F(1, 19) = 4.85$, $p < .05$) (Figure 2.1). This effect was driven by the females, as indicated by a significant Hand X Sex interaction ($F(1, 19) = 8.28$, $p < .01$). Follow-up paired t-tests indicated that females were better with the right hand ($t(10) = 3.32$, $p < .01$), while there was no significant hand difference in males ($t(9) = -0.54$, ns). Hand Use and Sex had no significant effect on latencies (Hand Use: ($F(1, 19) = .01$, $p = .92$), Sex: ($F(1,19) = 1.64$, $p=.22$)).

For the Hill test, the ANOVA revealed a significant effect of Hand Use on the scores ($F(1, 19) = 6.81$, $p< .02$), indicating a right hand advantage, independent of sex. The other effects were not significant (Sex: ($F(1, 19) = .02$, $p = .88$), Sex X Hand Use: ($F(1, 19) = .24$, $p = .63$). As with the Valley test, latencies were not significantly affected by Sex ($F(1.19) = .01$, $p = .92$) or Hand Use ($F(1,19) = .29$, $p = .60$).

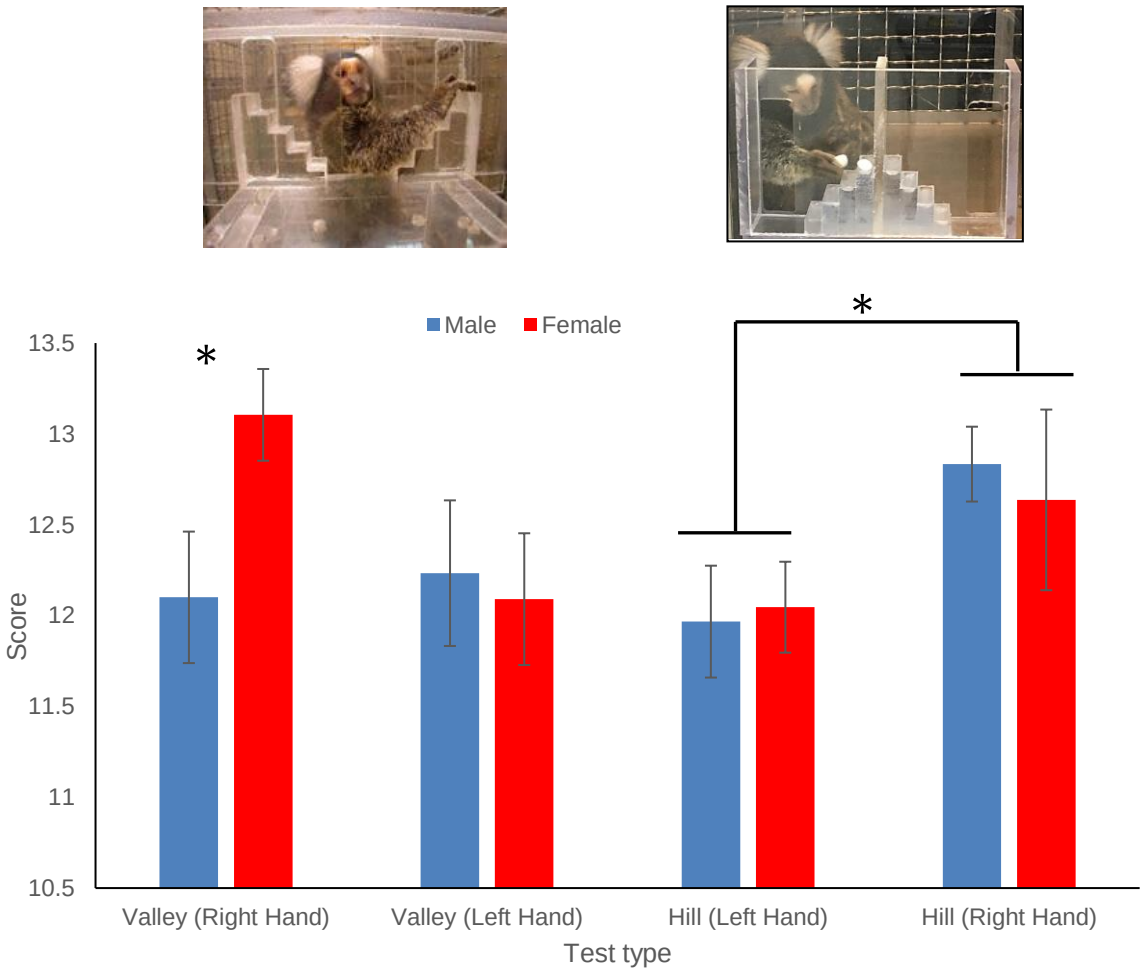

Fig. 2.1 Mean scores + SEM on Hill and Valley task separated by sex and hand used (apparatuses pictured above)

Discussion

We examined the effects of Sex, Handedness, and Hand Use on performance during the Hill and Valley motor task. While latency to complete the task was not affected by Sex, Handedness, or Hand use on either the Hill or Valley task, animals'

score on the task was affected by Sex and Hand Use. On the Valley test, female animals performed better with the right hand than with the left hand, independent of Handedness, while males performed similarly with either hand. On the Hill test, both female and male animals performed better with the right hand, again, independent of Handedness.

It is difficult to put these data in the context of other marmoset studies that use the Hill and Valley task. This task is usually used to understand the effects of experimentally induced neurodegeneration or stroke on motor function and as such, sex and hand performance differences are usually not included as variables in these studies. However, one study that used only adult male marmosets (2-5 yrs) did find greater variability in scores on the Valley compared to the Hill task (Phillips et al., 2017), which could indicate impaired performance in males when coordination between contralateral limb and visual field is required.

Hand preference had no measurable effect on Hill and Valley performance on our study. Hand preference in marmosets has been shown to be stable across time in several studies (Gordon & Rogers, 2015; Hook & Rogers, 2008), and most studies find equal distribution between Left hand preference, Right hand preference and ambidextrous marmosets, with no hand preference differences emerging at the group level (Cordeiro de Sousa et al., 2001; Hashimoto, Yamazaki, & Iriki, 2013; Piddington & Rogers, 2009). While hand preference in marmosets has been examined in terms of its effects on dual attentional task performance (Piddington & Rogers, 2009), emotional reactivity (Braccini & Caine, 2009), and cognitive bias (Gordon & Rogers, 2015), there is a dearth of information regarding the effects of hand preference on motor performance. Indeed, none of the recent papers employing the Hill and Valley task in marmosets have included

hand preference (Bihel et al., 2010; Eslamboli et al., 2003; Freret et al., 2008; Marshall et al., 2003; Phillips et al., 2017). Our study suggests that neither the Hill or Valley test induce a group level hand preference; however, it is possible that the training marmosets received with both hands before completing the test version the task washed out any potential effects of hand preference.

An interesting result of this study is the right hand advantage in performing the Hill task. Since the seminal work of Brinkman and Kuypers in rhesus macaques, it is well known that limbs are controlled by the contralateral brain hemisphere (Brinkman & Kuypers, 1973), suggesting that superior right hand performance in Hill for both sexes may be due to a left hemisphere advantage in motor coordination. This hypothesis is further supported by data in humans showing that planning of complex motor actions is lateralized to the premotor regions of the left hemisphere (Johnson-Frey, 2004).

The results from the Valley test are more difficult to interpret, as the right-hand advantage was shown only in females. Contrary to the Hill, the Valley test required monkeys to use the hand opposite to the visual field used. Thus, this indicated that the females were better using their right hand/left visual field in the Valley. Combining the results from both Hill and Valley, it follows that females show a *perceptual* advantage when using the left visual field, while both females and males appear to have a *motor* advantage for the right hand.

Experiment 2: Sex Differences in Stress Responsivity

Sex differences in the vulnerability to stress-related mental illnesses are well documented. Relative to men, women are more susceptible to a range of anxiety-related

disorders such as generalized anxiety disorder (Kessler et al., 1994), panic disorder (Sheikh, Leskin, & Klein, 2002), and Post Traumatic Stress Disorder (PTSD) (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995)(Bangasser & Valentino, 2014 for review). Among patients with anxiety-related disorders, women tend to experience more severe symptoms and are more negatively impacted by their illness than men (Altemus, Sarvaiya, & Neill Epperson, 2014). Despite these differences in disease susceptibility and severity, men and women are generally found to have similar basal levels of cortisol, the main stress hormone in primates (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). In contrast, cortisol responses to laboratory-induced stress have been found to be higher in young men than women in several different paradigms (Kajantie & Phillips, 2006; Kudielka & Kirschbaum, 2005 for review) and to vary as a function of the menstrual cycle in women (Kajantie & Phillips, 2006), with women having enhanced cortisol responses when estrogen levels are high. Thus, sex differences in cortisol levels are higher when women are in the follicular phase (Kirschbaum et al., 1999) and minimal or absent during the mid-luteal phase (Kirschbaum et al., 1999).

In rodent models, stress is known to affect cognitive performance differently in males and females. Female rat performance on some spatial tasks, such as the Y-maze (Conrad, Grote, Hobbs, & Ferayorni, 2003), and non-spatial tasks, such as object recognition memory (Bowman, Beck, & Luine, 2003), is unaffected by exposure to 21-day chronic restraint stress, while male performance on these tasks is impaired. Furthermore, female performance is *enhanced* by chronic restraint stress on other spatial tasks, such as the Morris water maze and the radial arm maze, while male performance is again impaired by the stressor (Bowman et al., 2003). It has been suggested that this

effect is due to a combination of both the organizational and activational actions of E2, as ovariectomized (OVX) female rats show no decrement in cognitive performance and an increase in cognitive performance in response to stress when E2 levels are restored (Bowman, Ferguson, & Luine, 2002; McLaughlin, Baran, Wright, & Conrad, 2005).

These data, which suggest a resilience to stress in females, are in contrast with human data showing an increased vulnerability to stress-related mental illnesses (Bangasser & Valentino, 2014). One way to clarify the complex relationship between stress and cognitive performance is to utilize NHPs, which are excellent models for the human stress response (Kalin & Shelton, 2003; Meyer & Hamel, 2014). In marmosets and other NHPs that form social bonds, mate separation can trigger robust Hypothalamic-Pituitary-Adrenal (HPA) activation and behavioral indications of stress (Cross, Pines, & Rogers, 2004). For example, social separation elicits reliable stress responses in marmosets.

French et al. studied HPA axis and behavioral responses to an 8 hour isolation in young marmosets (*Callithrix geoffroyi*) at 6, 12, and 18 months of age (French et al., 2012). While there was an age-related reduction of the cortisol response, no sex difference was observed at any age. We note that these monkeys were tested prior (6 months) during (12 months) and after (18 months) the onset of puberty, which usually occurs between 9-13 months in *Callithrix* (Abbott, Barnett, Colman, Yamamoto, & Schultz-Darken, 2003), but this study did not include older animals, so potential sex differences in adult animals could not be assessed. Johnson et al. (1996) used a longer separation paradigm (2 weeks) in common marmosets and reported that not only did adult females have significantly increased plasma cortisol levels compared to males in

response to separation, but females also had significantly higher cortisol levels in both the stressed and unstressed states.

Preliminary Work

In previous work from our lab, we showed that female marmosets have a more robust behavioral response to, and may take longer to recover from, a temporary social separation. Twelve gonadectomized (GDX) marmosets (6 female mean age = 5.7 yrs, 6 males mean age = 7.5 yrs) were exposed to a 7-hour social separation, in which the focal animal was removed from its home-cage and placed in a novel but identical cage in another room. Each animal had access to food and water but did not have visual or auditory access to its cage-mate or any other conspecific.

We compared behavior before the separation with behavior during the separation as well as baseline urinary cortisol with urinary cortisol the following morning. Female locomotor behavior significantly decreased during the separation ($Z = -2.20$, $p = .03$) while male locomotor behavior was not significantly affected ($Z = 1.59$, $p = .11$; Fig. 2.2). On the morning following the separation, female cortisol levels tended to be elevated compared to baseline (t

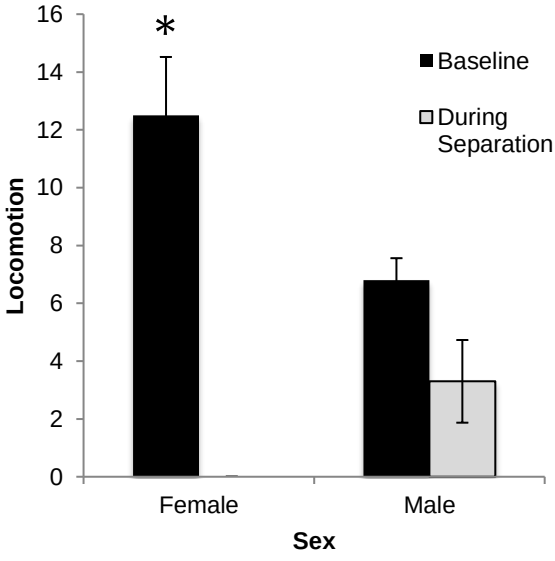

Fig. 2.2 Number of instances of locomotor behavior + SEM at baseline and during separation in males and females

(5) = 2.49, p = .055, d = .64). Male cortisol levels did not differ from baseline 16 hours after reunion with cage-mate (t (5) = 1.03, p = .35, d = .37; Fig. 2.3).

From these data, we see a difference in behavioral response and a potential difference in endocrine response to social stress between males and females. However, it is unclear how these results would differ in gonadally intact animals. Additionally, this experiment did not have data points assessing changes in cortisol during the separation phase.

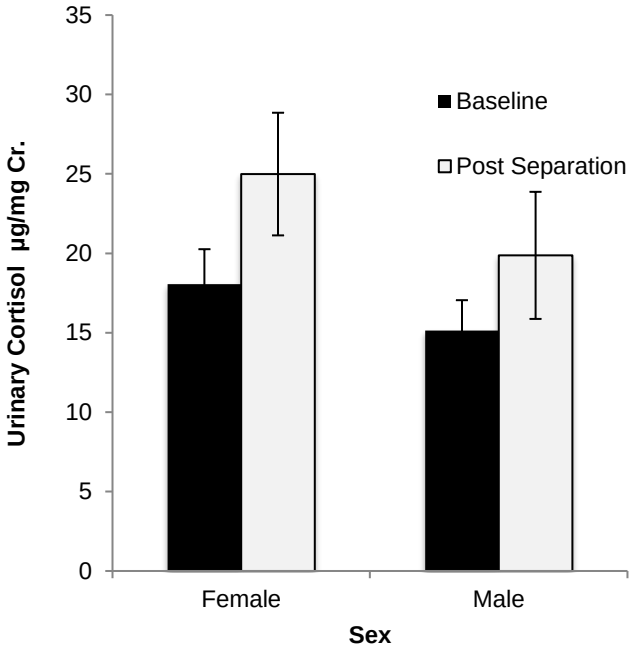

Fig. 2.3 Mean Urinary cortisol at baseline + SEM and at 16 hours post separation in males and females

In the present study, we examined urinary cortisol and behavioral indices of stress in response to social separation in intact, middle-aged marmosets of both sexes to determine whether sex differences in stress reactivity are present. Data were collected pre-separation, during separation, and post-separation. Based on our previous work in GDX animals, we predicted that females would show a greater increase in urinary cortisol and exhibit a more robust behavioral response to social separation than males.

Subjects

Twenty-eight marmosets ranging from 4 to 6 years old were used for this study (14 females, mean age = 4.94 years, SD = 0.68; 14 males, mean age = 4.73 years, SD = 0.52).

Social Separation

Following urine collection (approximately 7:30) and behavioral recording (approximately 8:30) (see below), the focal animal willingly entered a transport box and was immediately transferred from its colony room to an adjacent room with an identical cage. Separated animals had access to food and water *ad libitum*. Behavior was video recorded for later analysis (see below). Animals were reunited with their cage mates at approximately 16:30, after 7 hours had elapsed.

Behavioral Observations

Animals were video recorded at baseline (BL, approximately 30 minute prior to separation, 8:30), throughout the separation period, and 24 hour post-separation (PostSep, the day following separation, 8:30). All behavior was scored from the video recording by three experimenters with 90% interrater reliability. Reliability was assessed by dividing the total number of observations in agreement by the total number of observations. Behavior during separation was scored as follows: SST1 (first 5 minutes after experimenter left separation room), SST2 (5 minute sample 3.5 hours after start of separation), and SST3 (final 5 minute of 7 hour separation). Behaviors from the 3 time points were then averaged to create an overall separation score.

Behavior was measured using a modified frequency scoring system in which 20 behaviors of interest were recorded for the focal animal at 15-second intervals for five minutes. Behaviors included measures of locomotion, sociality, and aggression, adapted from an extensive ethogram developed for the marmoset (Stevenson, 1977; Appendix A). Marmosets exhibited very few stress behaviors other than altered locomotion patterns (Johnson et al., 1996). We recorded instances of both calm and agitated locomotion, with increased agitated locomotion being indicative of increased stress (see Appendix A).

Because of our previous results, which showed suppression of locomotor behavior in females during separation, we also quantified number of instances in which the animals actively scanned the environment while sitting still (inactive alert) and the amount of time animals spent sitting still with eyes closed (inactive rest).

Urine Collection and Assays

Urine samples were collected to assess cortisol levels in each animal immediately prior to separation (BL) and the morning following separation (post-separation), using a method described by Saltzman et al. (2004) (Saltzman, Prudom, Schultz-Darken, Wittwer, & Abbott, 2004). Briefly, animals entered the transport box at approximately 7:30; a few minutes after the lights turned on, and remained there until they urinated or until 30 min had elapsed. During the 7-hour separation, experimenters entered the separation room once each hour and collected any available urine from a catch pan underneath the animal's cage. Urine was pipetted into 1.5 ml vials, spun for five minutes and then frozen at -20^{o}C. The Endocrine BioServices Assay Lab at the University of Omaha, NE, USA, performed all urinary cortisol assays using enzyme

immunoassays (EIA) as described in French et al. (French et al., 1996). Briefly, samples were diluted in phosphate buffered saline (PBS), combined with β-glucuronidase and incubated at 37°C for 18 hours. Free and unconjugated steroids were extracted using anhydrous diethyl, evaporated in a hot water bath under nitrogen and reconstituted in PBS. The cortisol EIAs were performed using an antisera and a horseradish peroxidase (HRP) labeled cortisol conjugate. To control for differences in fluid intake and output, hormone concentrations were corrected using creatinine concentration.

Urine samples were grouped as follows: averaged samples from hours 1 and 2 of separation (SST1), averaged samples from hours 3, 4 and 5 (SST2), and averaged samples from hours 5 and 6 (SST3). Because we were only able to collect samples from a few animals in the first two hours of the separation, the analyses do not include SST1.

Statistical Analyses

Mixed measures ANOVAs were performed on the four behaviors of interest (calm locomotion, agitated locomotion, inactive rest, inactive alert) with sex and test phase (BL, SST1, SST2, SST3, PostSep) as factors. A mixed measures ANOVA was performed for cortisol levels with sex and test phase (BL, SST2, SST3, PostSep) as factors. To investigate the relationship between sex and test phase, we used a paired samples t test to compare BL to SST2, SST3, and PostSep cortisol levels in females and males. A bonferroni correction was used to correct for multiple comparisons. A p value of .017 was used to indicate a significant result.

Results

Behavior

For agitated locomotion, there was a marginally significant sex X test phase interaction ($F(2, 28) = 3.04$, $p = .06$, partial $\eta^2 = .18$), with females showing more agitated locomotion during separation than at BL ($p = .02$) or PostSep ($p = .06$). Males' agitated locomotor behaviour was unaffected by the separation (all p's > .05) (Fig. 2.4B).

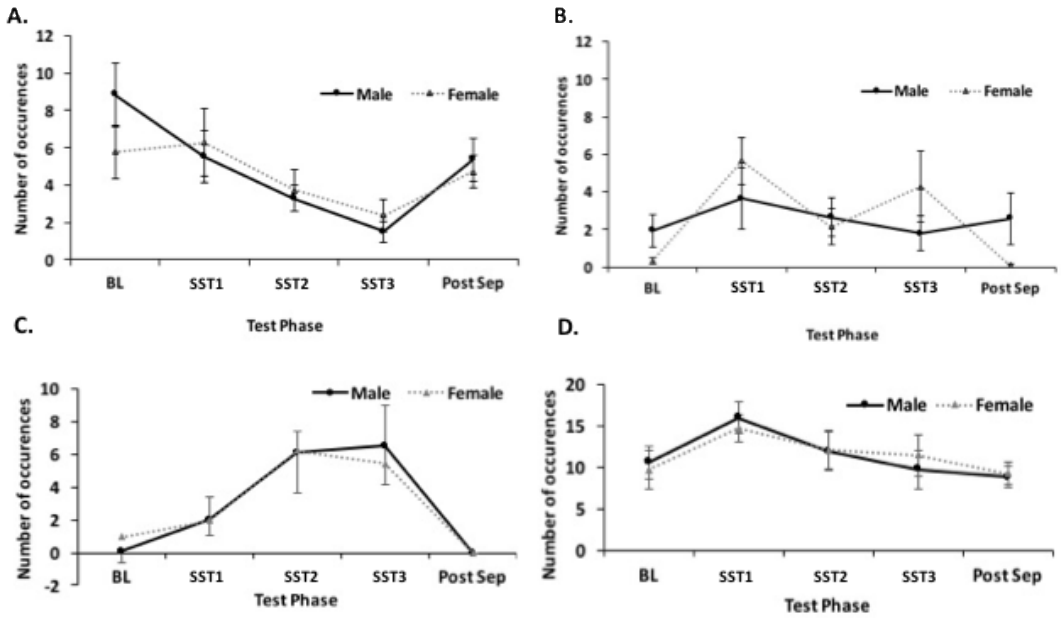

Fig. 2.4 Behaviors before, during, and after separation (means + SEM). A. Calm locomotion B. Agitated locomotion C. Inactive rest D. Inactive alert

While sex had no significant impact on calm locomotion, there was an effect of test phase ($F(2, 28) = 4.86$, $p = .015$, partial $\eta^2 = .26$), with a decrease in calm locomotion from baseline to separation ($p = .022$) for all animals (Figure 2.4A)

There were no effects of sex or test phase on inactive alert (all p's > .05) (Figure 2.4D), however, there was an effect of test phase on inactive rest ($F(2, 28) = 4.93$, $p = .015$, partial $\eta^2 = .26$), with more inactive rest during separation than at PostSep ($p = .02$) (Figure 2.4C) for all animals.

Cortisol levels

There was a significant effect of test phase on urinary cortisol levels ($F(3, 30) = 9.63$, $p < .001$, partial $\eta^2 = .49$), with an increase in cortisol from BL to SST2 ($p = .003$) and from BL to SST3 ($p = .006$) and a return to BL levels of cortisol ($p = .06$) (Fig. 2.5).

For females, cortisol levels significantly increased from BL at SST2 ($t(5) = 3.81$, $p = .013$) and at SST3 ($t(9) = 3.39$, $p = .008$), but returned to BL levels by the PostSep cortisol measurement ($t(11) = .77$, $p = .459$). In males, the increase in cortisol happens later, with levels similar to BL at SST2 ($t(6) = 2.04$, $p = .087$), significantly increased from BL at SST3 ($t(13) = 4.77$, $p < .001$) and returned to BL levels at PostSep ($t(13) = 1.82$, $p = .092$; Figure 2.5).

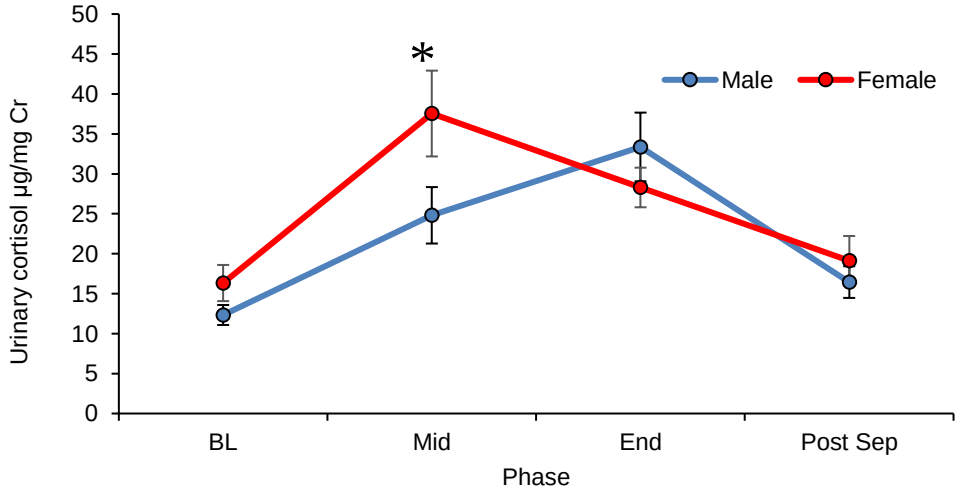

Fig. 2.5 Urinary cortisol (mean + SEM) before, during, and the morning following separation in males and females

Discussion

We examined whether stress reactivity to temporary social separation differs between the sexes in intact (vasectomized males) middle-aged marmosets (ages 4-6). While both sexes responded to the social stressor with a decrease in both calm locomotion and an increase in inactive rest, compared to males, females exhibited a significant increase in agitated locomotion during the stressor and an increase in cortisol levels that occurred earlier in the separation. The delay in the males' cortisol response suggests smaller acute reaction to the stressor which could be due to increased psychological response to the stressor over time, or a physiological effect in which male HPA response to stressor is delayed compared to females.

We found that intact females significantly increased their agitated locomotor behavior in response to the separation, while there was no significant change in locomotor behavior in males. This result is contrary to that of another social separation study in marmosets, which reported an increase in locomotor behavior in *both* sexes (Johnson et al., 1996). It is possible that this discrepancy in locomotor findings is due to methodological differences. In the Johnson et al. study, behavior observations of focal animals were performed live, whereas in our current study, all behaviors were scored using video tape. Scoring from a recording allowed us to accurately separate calm locomotion from agitated locomotion, an indicator of stress in marmosets.

Regarding the sex difference, organizational effects of androgens may permanently reduce anxiety in male marmosets. Indeed, there is limited and somewhat contradictory evidence that the organizational effects of sex hormones underlie some of the sex differences in stress reactivity. Specifically, one study found that neonatal

testosterone exposure in female mice partially masculinized patterns of response to an unpredictable stressor later in adulthood (Seney, Walsh, Stolakis, & Sibille, 2012). However, studies in rats have found inconsistent results. Rats GDX neonatally were found to be less anxious than controls in the elevated plus maze (Lucion, Charchat, Pereira, & Rasia-Filho, 1996; Zuloaga, Jordan, & Breedlove, 2011) as well as other anxiety tests (open field, novel object exposure, light/dark box; (Zuloaga et al., 2011)), suggesting anxiogenic effects of early testosterone exposure on adult behavior. In contrast, adult male mice with the testicular feminization mutation (Tfm) of the AR, which disables its function were found to be more anxious in the light-dark box and novel object tests compared to wild type males (Zuloaga, Morris, Jordan, & Breedlove, 2008). Similarly, Tfm male rats showed more indices of anxiety in the novel object test than wild type controls in another study (Zuloaga, Poort, Jordan, & Breedlove, 2011). Thus, the organizational effects of testosterone seem to have inconsistent effects on adulthood anxiety patterns, depending on the type of stressor, species and methodology.

Studies of the activational effects of E2 on anxiety in animal models are equally mixed. Evidence from rat studies suggests that administration of exogenous E2 in GDX animals has anxiolytic effects (Bowman et al., 2002; Frye & Walf, 2004), with E2 treatment increasing open field entries and time in the open arm of an elevated plus maze. However, a study in mice (Morgan & Pfaff, 2001) found opposite effects, with E2 treated animals decreasing open field entries and spending less time in the open arm of an elevated plus maze. These results are in concordance with a study in cynomolgus macaques (Stavisky et al., 2003) which found that exogenous E2 increased serum levels cortisol in GDX animals.

In our previous social separation experiment, we examined middle-aged marmosets (over 5 years old) that had been GDX in adulthood. We found that OVX females were more reactive to social separation than GDX males, as evidenced by a significant decrease in locomotor behaviors and range of behaviors during exposure to the stressor and an increase in cortisol levels nearing significance the day following separation. In contrast, the GDX males only exhibited a change in behavioral range, again suggesting that males may be less affected by the social stressor.

In our OVX study, females had a trend towards elevated levels of cortisol the morning following separation, whereas levels were not different from baseline in males. This is in contrast to our intact study, in which females showed an increase in cortisol earlier in the separation than intact males, but both females and males returned to baseline levels of cortisol by the morning following separation. This finding suggests a longer recovery phase in OVX females than GDX males, which was not seen in our intact sample. While this explanation is reflected in the data collected in our preliminary and current experiments, it should be verified in future studies with systematic control of these variables in which GDX and intact animals can be compared directly to one another.

A number of discrepancies characterize the literature regarding sex differences in stress reactivity in marmosets, with some studies finding females more reactive (de Sousa, de Menezes Galvão, Sales, de Castro, & Galvão-Coelho, 2015; Galvao-Coelho, Silva, & MB, 2012; Johnson et al., 1996), and others finding no sex difference (French et al., 2012; Pryce, Palme, & Feldon, 2002). The likely sources of variation for these findings are the age of the animals and the type and duration of stressor used. With

regard to age, several longitudinal studies in marmosets have characterized the developmental course of behavioral and cortisol response to stress. In general, juveniles show higher baseline cortisol levels than adults, with cortisol levels being progressively reduced from 6 to 16 months (de Sousa et al., 2015). However, in that study, cortisol levels were significant greater in females than males in older juvenile and subadult monkeys. In addition, juveniles also show greater stress responses and poorer post-stressor regulation than adult monkeys (French et al., 2012). Although French (2012) did not report sex differences, de Sousa et al., (2015) observed greater cortisol levels in response to a separation procedure in males than in females at 6 and 9 months, but the sex difference was no longer present at 12 months. In adult marmosets of unspecified age, Johnson et al. reported basal cortisol levels that were threefold higher in females than in males at baseline, and higher cortisol levels in females in response to social isolation (Johnson et al., 1996).

While we did not find any differences in basal cortisol levels in our intact animals, our results are consistent with this report in that the intact females had a more robust behavioral response and an earlier rise in cortisol during the stressor than intact males.

Experiment 3: Sex Differences in Cognitive Performance

There are widely accepted sex differences in cognitive performance. Men tend to outperform women on spatial rotation (Voyer et al., 1995) and navigational tasks (Astur et al., 1998; Chai & Jacobs, 2009; Driscoll et al., 2005; Persson et al., 2013; Sandstrom et

al., 1998). Women show superior performance in verbal fluency (Heinzel et al., 2013), verbal memory (Munro et al., 2012; Murre et al., 2013), and memory for object locations (Barel, 2016; Duff & Hampson, 2001; Honda & Nihei, 2009; Lejbak et al., 2009). Despite this evidence, some studies have called into question the magnitude of these sex difference, as differences have been shown to be affected by socioeconomic status (Levine et al., 2005) and gender equity in a participant's country of origin (Lippa et al., 2010). The potential impact of these socio-cultural factors highlights the importance of examining cognitive sex differences in an appropriate animal model. We propose the use of the marmoset for such a model.

Marmosets have sophisticated cognitive skills. They can use cognitive maps in spatial memory tasks (MacDonald, Pang, & Gibeault, 1994), they show object permanence and demonstrate social learning (Huber et al., 2009). In addition, marmosets can be trained to use tools (Yamazaki et al., 2011) and, unlike the rhesus monkey, are able to imitate conspecific demonstrators (Voelkl & Huber, 2000). They can also perform more standardized cognitive tasks administered in the Wisconsin General Testing Apparatus (WGTA) and have been successful in a range of prefrontal-dependent tasks, such as the Delayed Response (working memory) (Lacreuse et al., 2014; Miles, 1957), reversal learning (cognitive flexibility) (Lacreuse et al., 2014; Ridley, Haystead, & Baker, 1981) or detour reaching task (inhibitory function) (Lacreuse et al., 2014), as well as tasks more strongly dependent on the hippocampus, such as the Delayed Matching-to-Position task (Lacreuse et al., 2014).

Similar tasks can also be administered on computerized touch-screen systems. Roberts et al. (Roberts, Robbins, & Everitt, 1988) and later Spinelli et al. (Spinelli et al.,

2004) have extensively described marmoset performance on CANTAB, a touch-sensitive cognitive battery incorporating a wide range of tests, that has also been used to assess human (Robbins et al., 1994) and rhesus monkey (Weed et al., 1999) cognition. Custom-designed touch-screen batteries have also been successfully implemented for marmosets more recently (Kangas & Bergman, 2017; Takemoto et al., 2015; Yamazaki, Saiki, Inada, Watanabe, & Iriki, 2016).

Based on these data, marmosets can perform of wide range of cognitive tests. However, Spinelli notes that learning of visual (non) matching-to-sample tasks is challenging for marmosets (Spinelli et al., 2004; but see Nakamura et al., 2018). Despite this potential limitation, based on a comparative analysis of the Transfer Index, an index of reversal learning performance, Strasser et al. conclude that marmosets' cognitive performance is better than what would be expected for their brain size and superior to that of a larger New World monkey, the capuchin (Strasser & Burkart, 2012).

Only one study so far has investigated whether marmosets show sex differences in cognitive function. In this report, no sex difference was found in a group of 35 young marmosets (1-4 years old) performing visual discriminations and reversal tasks (Takemoto et al., 2015). However, it is unclear whether this pattern of performance is maintained with age and observed with more complex tasks.

Preliminary Studies

My first preliminary study demonstrates that estrogens affect selective aspects of cognition in females. We tested 11 OVX females implanted with Silastic capsules filled with E2 (E2, n=6) or empty capsules (controls, n=5) on a battery of cognitive tasks

assessing PFC dependent cognition (Object Reversals, OR; Delayed Response, DR) and hippocampal (HPC) dependent cognition (Delayed Matching-to-Position, DMP). E2 levels were similar to those during mid follicular phase of menses.

For the OR, monkeys were presented with a pair of three-dimensional objects, a white sphere and a black star, randomly placed over the left or right lateral wells of a testing tray, one of which hid a reward (freeze dried mini marshmallow). During the training phase (initial discrimination), which occurred prior to OVX, the black star was always rewarded. Monkeys had to select the black star to find the reward until they reached a criterion of 90% correct responses over two consecutive sessions (maximum of two errors in 20 trials). The test condition (object reversals) occurred 4 weeks after OVX and treatment. Here, the black star was no longer rewarded and the white sphere now hid the reward. Animals were retested for 10 trials per day until they reached a learning criterion of 90% correct responses over two consecutive sessions. Animals received a total of three reversal sessions.

In the DR task, monkeys observed the experimenter bait one of two lateral wells with a food reward and cover the wells with identical stimuli (opaque tokens). The tray was concealed from view for a specific delay and then re-presented to the monkey. The monkey had to select the token covering the reward. We used a procedure based on that employed by Collins et al. in marmosets with delays of 0, 1, 3, 6 and 10 s (Collins, Wilkinson, Everitt, Robbins, & Roberts, 2000). Monkeys were tested 10 trials per day, 5 days a week. Training (preoperatively): monkeys were trained to a 90% correct responses learning criterion on each successive delay. Testing (post-OVX and treatment): monkeys were tested with all delays mixed in a single session (two trials per delay, 10 trials per session) for a total of 100 trials, over 10 days of testing.

Unlike the previous tasks, the acquisition of the DMP was performed post OVX and treatment. In the task, monkeys were first presented with one opaque token over one of four wells. The tray was concealed from view for 1 s, after which the tray was re-presented to reveal the sample token over the same well and an identical token over a different well. The animal had to displace the token in the original location to retrieve the food reward. All four positions were used and the position of the token at each trial was pseudorandomized. Subjects were tested for six trials per day, 5 days per week, until a learning criterion of 90% correct responses over two consecutive sessions (12 trials) or a maximum of 350 trials.

Compared to controls, E2-treated monkeys tended to perform worse during the acquisition of the reversals, as they committed significantly more errors in the second reversal and showed an increase in perseverative responding from Reversals 1 to 3 (Figure 2.6A, 2.6B). In the DR, there was a marginally significant main effect of treatment ($F(1, 9) = 3.90$, $p = 0.08$), but the large effect size ($f^2 = 0.66$) suggested that the E2 group might be impaired relative to the control group **(**Figure 2.6C**)**. Delay significantly decreased performance ($F (4, 36) = 19.57$, $p < 0.001$), but the Delay x Treatment interaction was not significant.

In contrast, there was a marginal effect of treatment for the DMP task, indicating that E2 treated animals tended to outperform the controls on this task. All 6 monkeys in the E2 group but only 3 of the 5 monkeys in the control group learned the task within 350 trials. In addition, monkeys in the E2 group tended to learn the task in fewer trials than monkeys in the control group ($t (9) = -1.38$, $p = .10$, one-tailed; effect size Cohen's $d = 0.83$) and tended to make fewer errors than the control group (110 ± 29.61; $t (9) = 3.12$, $p = .09$; one tailed, $d = 0.86$; Figure 2.6D**)**. Despite the non-significant findings, the large

effect sizes obtained in the DR and DMP suggest functionally significant differences between the control and E2-treated groups.

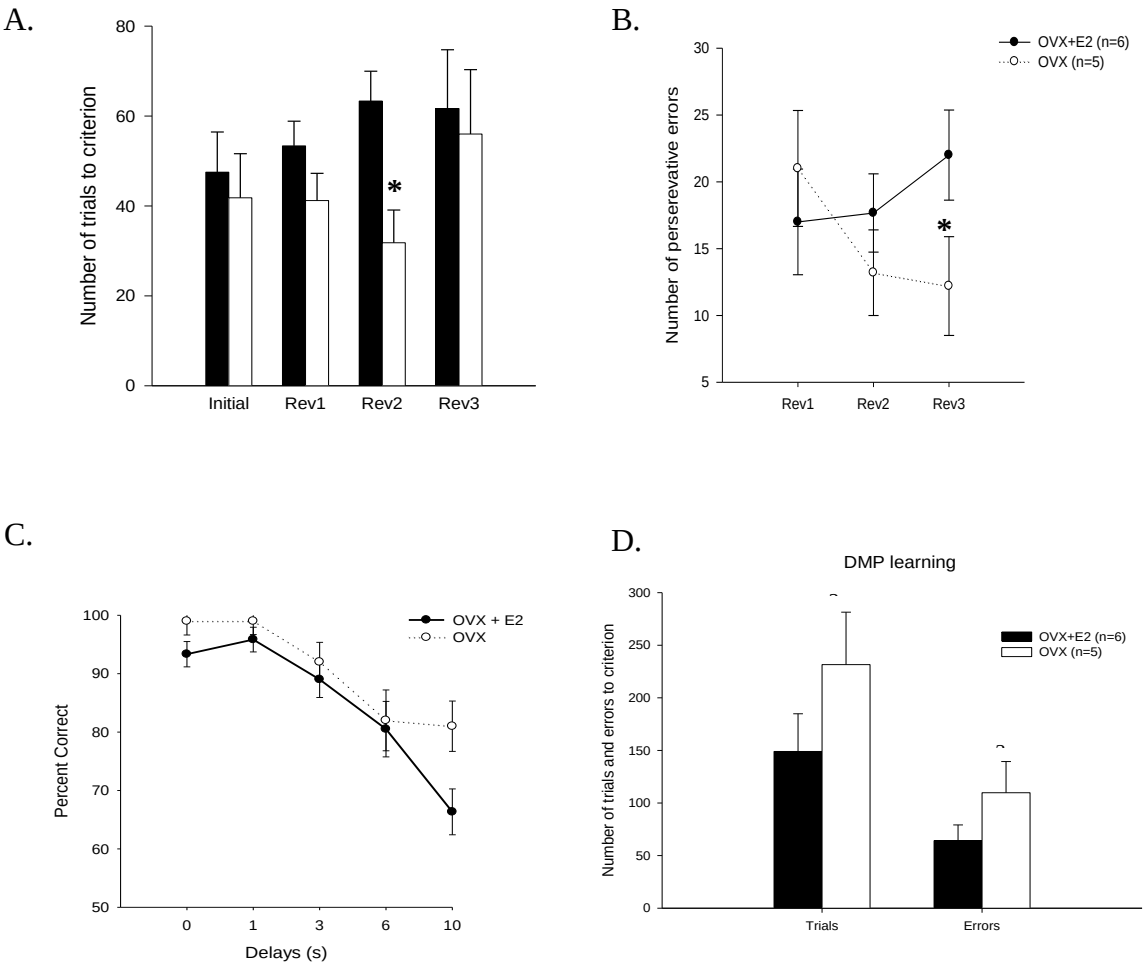

Fig. 2.6 A) Trials to criterion on OR task B) Perseverative errors for 3 reversals C) Percent correct on DR task D) Trials to criterion on the DMP task

My second preliminary study shows that female and male GDX marmosets have different response latencies on an object reversal task. Ten castrated male (mean age 5.5 years, 3.53 – 8.64) common marmosets were used in this experiment. Their performance was compared to that of 11 OVX females (mean age 3.7 years, 2.4 – 4.82) used in our previous study. Five males received weekly injections of testosterone cypionate (T group, 1.4 mg/kg) mixed with cottonseed oil and five animals received injections of oil vehicle (Control group). As stated above, 6 females were implanted with E2 capsules and 5 were implanted with empty capsules.

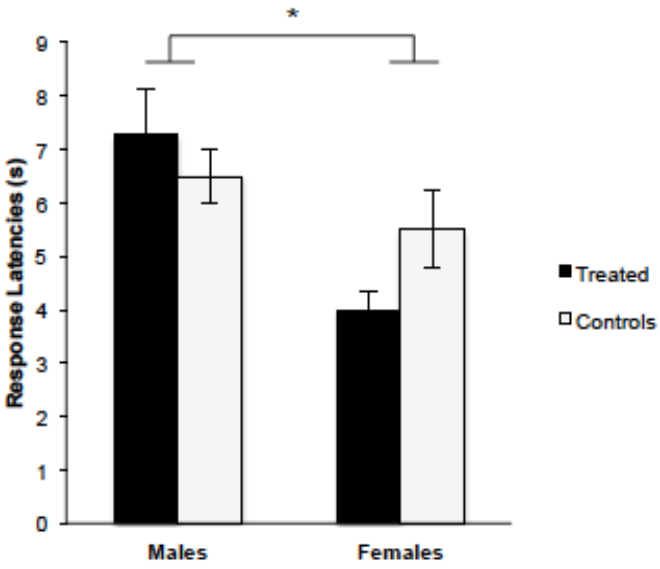

Fig. 2.7. Response latencies (mean + SEM) in sex hormone treated and untreated males and female

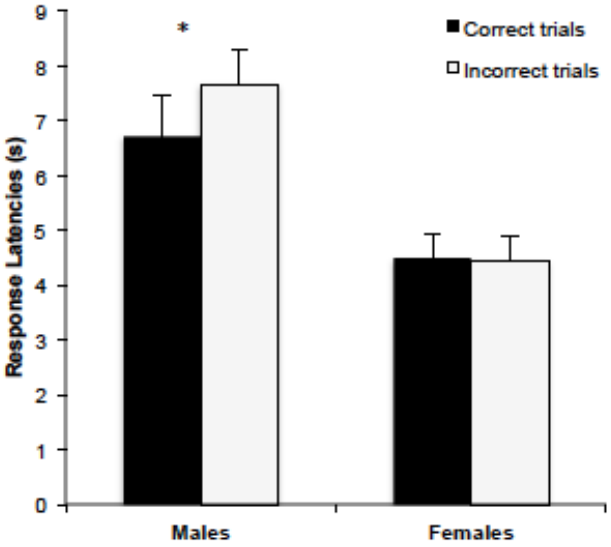

Fig. 2.8. Response latencies (mean + SEM) on correct and incorrect trials in males and females

There was no effect of T treatment in males (Reversal x Treatment, F (3, 24) = .64, p = .6). In terms of sex differences, a mixed measures ANOVA showed that there was no effect of Sex (F (1, 19) = .79, p = .42), Reversal (F (2, 38) = .99, p = .38), or Sex x Reversal interaction (F (2, 38) = .23, p = .79) on the number of trials to reach criterion. When we examined response latencies, we found that males (m = 7.2 $\pm$ .68 SEM) had

significantly longer latencies than females (m = 4.47 $\pm$ .65 SEM), independent of hormone replacement status (F (1, 16) = 6.97, p = .02; Figure 2.7).

We further analyzed response latencies by examining whether or not the animal was correct or incorrect on the trial. Males took significantly longer to respond on trials when they were incorrect (m = 7.67 $\pm$ .79 SEM) than on trials when they were correct (m = 6.72 $\pm$ 1.08 SEM), while female response times were not significantly affected by trial outcome (correct: m = 4.49 $\pm$ .60 SEM, incorrect: m = 4.45 $\pm$ .65 SEM); (Outcome x Sex: F (1,16) = 6.98, p = .02; Figure 2.8). Additionally, while all animals were significantly slower if they had been incorrect on the previous trial (F (1, 17) = 12.01, P = .003), there was a significant Sex × Previous Trial Outcome interaction, indicating male response latencies were more affected when the previous trial was incorrect, as opposed to correct (F (1, 17) = 9.78, P = .006; correct: t(19) = 1.97, P = .062), incorrect: t(19) = 2.36, P = .03). Overall, slower response latencies in males than females during Reversal Learning, especially during and following an incorrect trial, may reflect greater performance monitoring and inhibition in males compared to females in cognitive flexibility.

Based on these preliminary studies, we hypothesized that females would have an impairment in Reversals and ID/ED, reflected by an increase in both trials and errors to learning criterion. We predicted that females would take more trials and make more errors to reach learning criterion than males on both the Reversal task and the ID/ED.

Subjects

Twenty-two marmosets ranging from 4 to 6 years old completed the Reversals (11 females, mean age = 5.05 years, SD = 0.59; 11 males, mean age = 4.69, SD = 0.46). Out of these 22 subjects, 17 marmosets completed the ID/ED (10 females, mean age = 5.10 years, SD = 0.71; 7 males, mean age = 4.97 years, SD = 0.32).

Procedures

Monkeys were tested on the CANTAB, an automated cognitive testing battery used with humans (Robbins et al., 1994), and NHPs, including marmosets (Roberts et al., 1988; Spinelli et al., 2004).

Testing Apparatus

The nonhuman primate version of the CANTAB (Monkey CANTAB Intellistation with Liquid Reward, Model 80951A) consisted of a touch screen panel (37.78 cm) in a stainless-steel frame (56 x 38 x 30 cm) using an Intel based 1.6 GHz CPU operating system (Figure 2.9). A stainless-steel sipper tube in the middle of the screen delivered the reward (banana milkshake) via a peristaltic pump, at a rate of 0.2 ml per second.

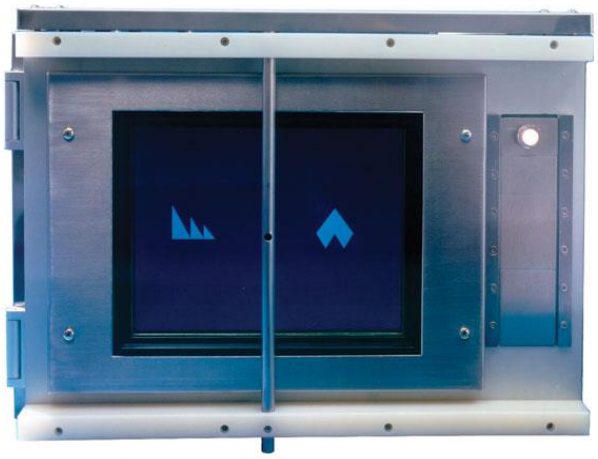

Fig. 2.9 CANTAB system displaying stimuli from the Simple Reversal Learning task

To encourage participation, food and water was removed from the animals' cages 2 hours prior to testing and replaced in the cage no later than 5 hours after removal. For testing, marmosets voluntarily entered a transport box attached to the front of their homecage. The CANTAB was positioned against the meshed front (2.5 x 2.5 cm openings) of the transport box, so animals could reach through to touch the screen and lick the reward from the sipper tube. Experimenters loaded CANTAB testing programs remotely from a desktop computer located outside of the marmoset housing rooms.

Tasks

CANTAB training

We followed the procedures described by Roberts et al. (a C. Roberts et al., 1988) and Pearce et al. (Pearce, Crofts, Muggleton, & Scott, 1998) for stages of tone-reinforcement associations and touch-training. Monkeys were trained to lick the milkshake from the spout, to associate a tone (41 Hz) with reward delivery (5 sec), to touch the screen, touch a large static square at the center of the screen and touch smaller

squares appearing successively at random locations on the screen, before being presented with the first pair of stimuli.

Discrimination and Reversal Learning

The marmosets were presented with a task assessing cognitive flexibility, reversal learning, which requires the ability to flexibly adapt to changing stimulus/reward contingencies. Previous research in marmosets had identified the orbitofrontal cortex (OFC) as the main brain area involved in this ability (Clarke, Robbins, & Roberts, 2008; Dias, Robbins, & Roberts, 1996a). For the task, marmosets were presented with a total of three pairs of stimuli depicted in Figure 2.10A. The first pair of stimuli consisted of a blue triangle and a white line. The second pair consisted either of 2 different white lines or two different pink shapes (the order of presentation of pairs 2 and 3 was counterbalanced between monkeys). For each pair, monkeys had to perform a simple discrimination (SD), followed by a simple reversal (SR). The two stimuli appeared in any position on the touch screen. Animals were given 40 trials a day to learn the stimulus/reward contingencies (for example, blue triangle always rewarded). Once they reached a 90% correct criterion, the stimulus/reward contingencies were reversed (e.g., the white line now rewarded). When the 90% accuracy criterion was reached on the SR, the marmoset moved on to a new stimulus pair. The number of trials to reach criterion (TTC) errors to reach criterion (EC) and response latencies (RL) on each trial were recorded. Using the procedure described in Lai et al., for each 40 trial session we calculated number of perseverative errors (when number of errors was significantly above chance 27-40), chance errors (number of errors when animals were performing at chance levels: 14-26), and learning errors (when the number of errors was significantly

below chance: 0-13) (Lai, Moss, Killiany, Rosene, & Herndon, 1995). In addition, the number of refusals (number of trials that the monkey refused to perform) was also recorded as an index of motivation.

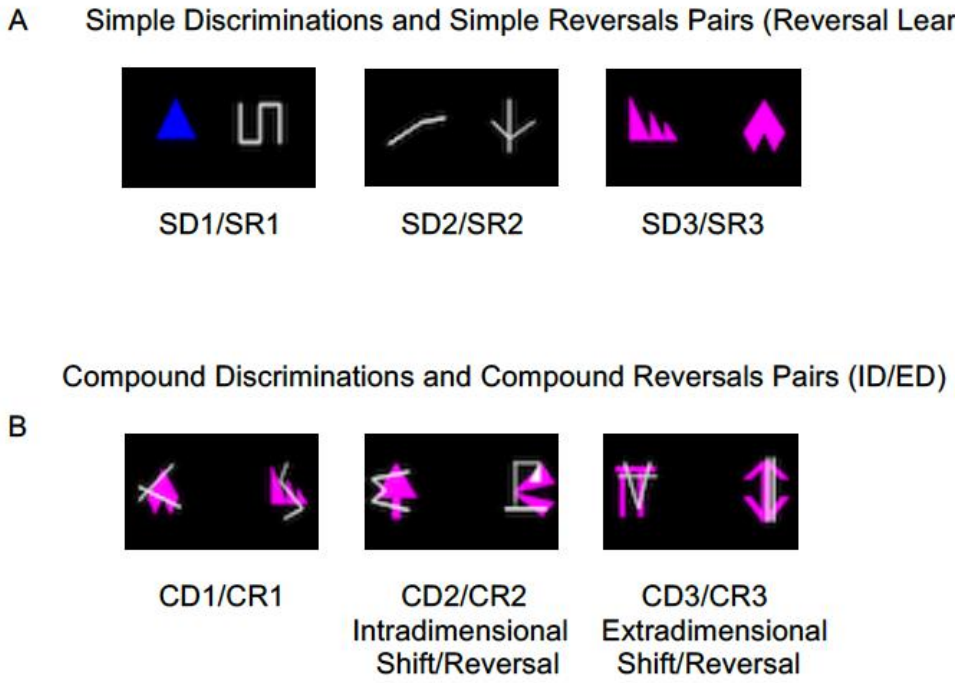

Figure 2.10 Examples of stimuli from Simple Reversal Learning (A) and from the ID/ED (B)

ID/ED

The ID/ED is a more complex task that incorporates both reversal learning and attention set shifting, which requires animals to shift from one set of stimuli dimensions to another. It uses compound stimuli that vary in 2 dimensions (i.e., shape and line). For the ID shift, the subject is required to continue to use the same dimension (e.g, shape) from the previous set of stimuli for a new set of discriminations involving new stimuli. For the ED shift, the subject must use the previously irrelevant dimension (lines) to be rewarded. Within each type of shift, the animals also perform reversal between the two

stimuli of the pair. Discrete lesions to different parts of the marmoset PFC produce dissociable effects on ED and reversals. Lateral PFC lesions produce deficits in ED shifting (Birrell & Brown, 2000) but have no effect on reversal learning, while integrity of OFC is required for reversal learning (Dias et al., 1996a).

For the ID/ED, the marmosets were presented with a total of three pairs of stimuli, however, each stimulus was a compound stimulus, consisting of a shape and a line, overlaid on top of one another (see Figure 2.10B). For the first discrimination (CD1) animals were given the exact same stimulus/reward contingencies as SR3 (e.g., same shapes and same rewarded stimulus as SR3), but with the addition of an extraneous dimension (e.g. lines) that they needed to ignore. It was followed by a reversal (CR1) in which they had to select the alternate shape of the pair. Animals were given 40 trials per day and were required to reach a 90% accuracy criterion to move on to the next stage of testing.

Intra-dimensional Shift (Pair 2, CD2/CR2): the second pair consisted of new stimuli, but the target dimension (e.g., shape) from CD1/CR1 continued to apply (shape rewarded dimension, lines ignored).

Extra-dimensional Shift (Pair 3): the final pair consisted of new stimuli, but this time monkeys had to switch from using the previous rewarded dimension (e.g, shape), to using the alternate dimension (e.g., line).

As for Reversal Learning, the number of trials to reach criterion (TTC) errors to reach criterion (EC) and response latencies (RL) on each trial were recorded. Perseverative, Chance, and Learning errors were calculated as described above. In

addition, the number of refusals (number of trials that the monkey refused to perform) was also recorded as an index of motivation.

Statistical Analyses

For the Reversals, the TTC, EC, and refusals were analyzed using a mixed ANOVA with Sex, Pair Number (Pair 1, Pair 2, Pair 3), and Test Type (SD, SR) as factors. The order of presentation of Pairs 2 and 3 (white lines or pink shapes first) was entered as a covariate in the analyses. RL was analyzed using a mixed ANOVA with Sex, Pair Number (Pair 1, Pair 2, Pair 3), and Test Type (SD, SR), and Outcome (correct trials vs incorrect trials) as factors. As Test Type had no effect on RL and did not significantly interact with any of the other factors, it was removed from subsequent analyses.

For the ID/ED, the TTC, EC, and refusals were analyzed using a mixed ANOVA with Sex, Pair Number (Pair 1, Pair 2, Pair 3), and Test Type (SD, SR) as factors. The order of stimulus presentation (Line, Line, Shape or Shape, Shape, Line) was entered as a covariate in the analysis. RL was analyzed using a mixed ANOVA with Sex, Pair Number (Pair 1, Pair 2, Pair 3), and Test Type (SD, SR), and Outcome (correct trials vs incorrect trials) as factors. Out of the 17 subjects, 2 males and 1 female did not complete CR3 within 3000 trials. For two of these animals (1 female and 1 male), the maximal trial completed (3000) was used as TTC value. The third male was still performing at 50 % (chance) after 2191 trials but did not reach 3000 trials within the time allotted for data collection. For this animal, we used multiple imputation to calculate 10 estimated values for each dependent variable of interest (TTC, EC, RL, Nonresponse). These values were

then averaged for each parameter the averaged value used in the subsequent analyses. Imputed values can we seen in Appendix B.

Results

Reversals

Trials to Criterion (TTC)

The ANOVA revealed a significant main effect of Test Type ($F (1, 19) = 64.86$, $p < .001$, partial $\eta^2 = .77$) on TTC, with animals taking significantly more trials to learn the SRs (m = 448.93, SEM = 37.51) than the SDs (m = 224.24, SEM = 22.20**)**. Additionally, Pair Number was also significant ($F (2, 38) = 21.15$, $p <.001$, partial $\eta^2 = .53$) with animals taking significantly fewer trials on the 1st pair (m = 159.37, SEM = 15.48) than on the 2nd (m = 386.48, SEM = 36.88) and 3rd (m = 663.91, SEM = 50.01). The main effect of Sex on TTC was not significant ($F (1, 19) = .40$, $p = .54$, partial $\eta^2 = .02$), however, a significant interaction between Sex and Test Type ($F (1, 19) = 7.93$, $p = .01$) revealed that females needed more trials m = 496.66, SEM = 53.17) than males (m = 401.22, SEM = 53.17) to reach criterion on the SRs, but not on the SDs (Males: m = 235.16, SEM = 31.46, Females: m = 213.33, SEM = 31.46, Figure 2.11). A significant interaction between Test Type and Pair Number ($F (1.26, 23.88) = 7.12$, $p = .009$, partial $\eta^2 = .27$) also indicated that monkeys had higher TTC for SRs than SDs on all three pairs (all p's < .001). Finally, a marginal Sex X Test Type X Pair Number ($F (1.26, 23.88) = 3.00$, $p = .088$, partial $\eta^2 = .14$) suggested that females were especially impaired for the more complex pairs.

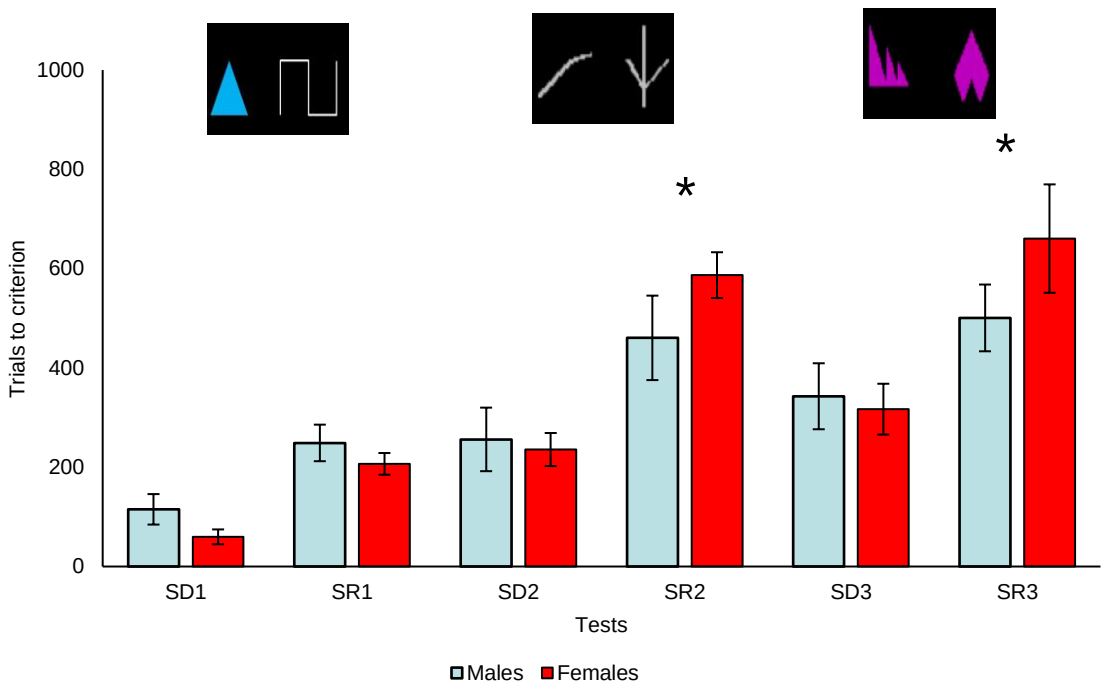

Fig. 2.11 Mean trials to criterion + SEM on the Simple Reversal learning task in males and females; examples of stimuli shown above

Errors to Criterion (EC)

There was a significant main effect of Test Type ($F (1, 19) = 60.62$, $p < .001$), with animals making significantly more errors during SRs (M = 210.38, SEM 17.45) than SDs (M = 76.74, SEM = 7.47). There was also a main effect of Pair Number ($F (2, 38) = 18.30$, $p < .001$) with animals making significantly more errors on pair 2 (M = 157.5, SEM = 15.04) and pair 3 (M = 190.25, SEM = 20.11) than on pair 1 (M = 82.93, SEM = 7.04). A marginal Sex X Test Type X Pair Number ($F (2, 38) = 2.61$, $p = .087$, $\eta^2 = .121$) indicates that males (M = 51.93, SEM = 8.05) made more errors than females (M = 23.62, SEM = 8.05) on only the first discrimination (SD1, $p = .023$).

Perseverative Errors

On the three SRs, there were no significant effect of sex ($F(1, 20) = .005$, $p = .95$, partial $\eta^2 < .001$), no significant effect of reversal number ($F(2, 40) = .12$, $p = .89$, partial $\eta^2 = .006$) and no significant interactions sex X reversal number ($F(2, 40) = 1.21$, $p = .31$, partial $\eta^2 = .06$).

Response Latencies (RL)

There was a significant effect of outcome on RL ($F(1, 20) = 5.17$, $p = .03$), with animals taking significantly longer on incorrect trials (m = 2924.33 ms, SEM = 92.94) than on correct trials (2884.23 ms, SEM = 100.32 ms). There was also a significant main effect of test number, with animals taking significantly longer to respond on the first pair (m = 3140.08 ms, SEM = 93.49 ms) than on the second (m = 2883.83 ms, SEM = 107.68 ms) or the third (m = 2752.44 ms, SEM = 121.39) pairs. There was no significant effect of sex on RL ($F(1, 20) = .000$, $p = .99$) and no significant interactions (sex X outcome ($F(1, 20) = .59$, $p = .45$), sex X number ($F(2, 40) = .05$, $p = .96$), sex X outcome X number ($F(2,40) = 1.09$, $p = .35$)).

Non-Responses

Monkeys refused more trials during SRs (m = 366.02, SEM = 64.59) than during SDs (m = 154.39, SEM = 25.17), as indicated by a significant main effect of Test Type ($F(1, 19) = 5.87$, $p = .03$, partial $\eta^2 = .24$). There was also a significant effect of Test

Number (F (2, 38) = 3.19, p = .05, partial η^2 = .14), indicating that monkeys refused more trials for Pair 3 (m = 463.91, SEM = 50.01) and 2 (m = 386.48, SEM = 36.88) than Pair 1 (m = 159.39, SEM = 15.48). Importantly, sex did not affect the non-responses (p = .50) and did not interact with Test Type (p = .91) or Test Number (p = .99).

ID/ED

Total Trials to Criterion (TTC)

The ANOVA revealed a significant main effect of Test Type (F (1, 14) = 119.73, p < .001, partial η^2 = .90), with animals taking significantly more trials to learn the CRs (m = 963.35, SEM = 60.79) than the CDs (m = 513.43, SEM = 42.26,); however a significant Test Type X Pair Number interaction (F(2, 28) = 8.11, p = .002, partial η^2 =

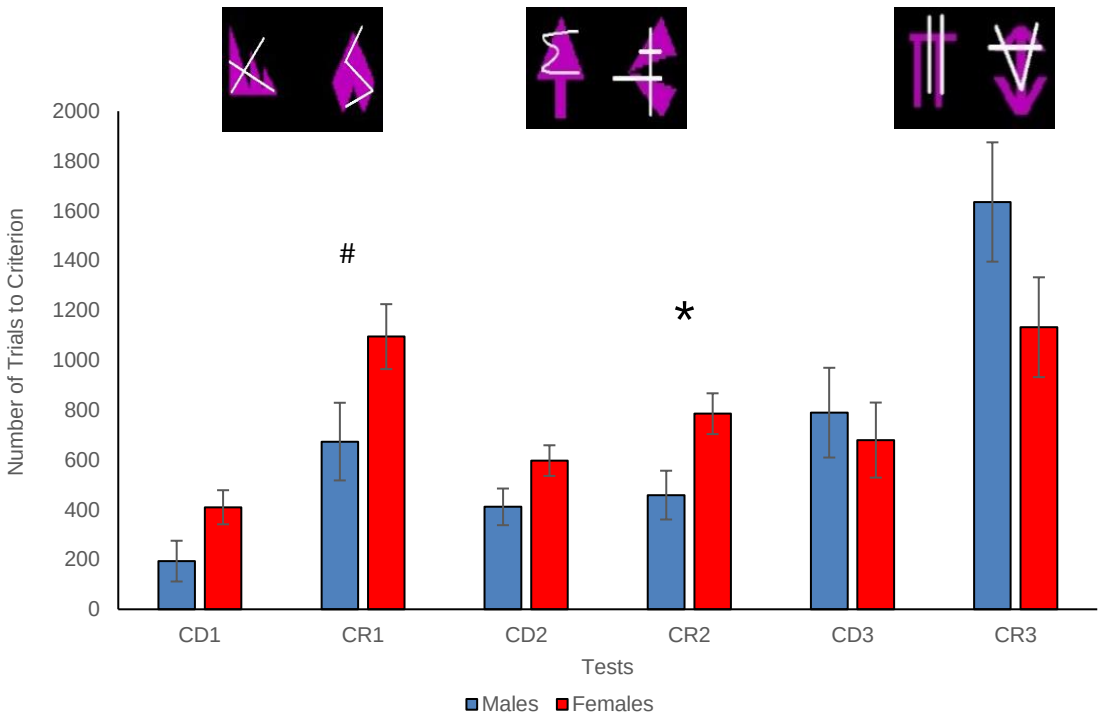

Fig. 2.12 Mean trials to criterion + SEM on the ID/ED in males and females (# indicates marginal significance (p = .057)); examples of stimuli shown above

.37) indicated that animals only needed significantly more trials for CRs on pair 1 and pair 3. Additionally, Pair Number was also significant ($F (2, 28) = 26.28$, $p < .001$, partial $\eta^2 = .65$) with animals taking significantly fewer trials on Pair 1 (m = 592.86, SEM = 69.14) and pair 2 (ID shift and reversal: M = 563.06, SEM = 41.31) than pair 3 (ED shift and reversal: m = 1059.25, SEM =125.58). The main effect of Sex on TTC was not significant ($F (1, 14) = .87$, $p = .37$, partial $\eta^2 = .06$), however, a significant interaction between Sex and Pair Number ($F (2, 28) = 3.84$, $p = .03$, partial $\eta^2 = .22$) revealed that females needed more trials to reach criterion than males on pair 1 (Females: m = 752.32, SEM = 88.81; Males: m = 433.4, SE = 106.2; $t(15) = 2.37$, $p = .03$) and pair 2 (Females: m = 691.20, SEM = 53.07; Males: m = 434.93, SEM = 63.45, $t(15) = 2.49$, $p = .03$) but not on pair 3 (Females: m = 906.04, SEM = 161.32; Males: m = 1212.46, SEM = 192.90, $t(15) = .94$, $p = .36$). Finally, a marginal Sex X Test Type X Pair Number ($F(2, 28) = 2.58$, $p = .093$, partial $\eta^2 = .16$) suggested that females were particularly impaired on CR2 (ID reversal; Females: m = 785.33, SEM = 81.90; Males: m = 458.52, SEM = 97.93, $p = .02$; Figure 2.12), and performed marginally significantly more poorly than males on CR1 (Compound reversal; Females: m = 1094.87, SEM = 130.38; Males: m = 458.52, SEM = 97.93, $p = .057$).

Errors to Criterion (ETC)

There was a significant effect of Test Type ($F(1, 14) = 64.37$, $p < .001$, partial $\eta^2 = .82$) with animals making more errors during the CRs (m = 426.95, SE = 32.85) than the CDs (m = 187.44, SE = 15.65). There was also a significant effect of Pair Number ($F(2, 28) = 21.17$, $p < .001$, partial $\eta^2 = .60$), with animals making more errors on Pair 3

(ED shift/reversal; m = 420.81, SE = 51.39) than on Pair 1 (m = 255.52, SE = 30.57) or Pair 2 (ID shift/reversal; m = 245.26, SE = 21.69). There was not a significant main effect of sex ($F(1, 14) = 1.91$, $p = .12$, partial $\eta^2 = .16$) nor did sex interact with Test Type ($F(1, 14) = .78$, $p = .39$, partial $\eta^2 = .05$) or with Pair Number ($F(2, 28) = 2.35$, $p = .11$, partial $\eta^2 = .14$). The sex X Pair Number X Test Type interaction was also not significant ($F(2, 28) = .40$, $p = .68$, partial $\eta^2 = .03$).

Perseverative Errors

On the three CRs, there were no significant effect of sex ($F(1, 14) = 2.71$, $p = .12$, partial $\eta^2 = .16$), no significant effect of reversal number ($F(2, 28) = 1.60$, $p = .22$, partial $\eta^2 = .10$) and no significant interactions sex X reversal number ($F(2, 28) = .36$, $p = .70$, partial $\eta^2 = .03$).

Response Latencies (RL)

As with the reversals, there was a main effect of outcome on RL for the ID/ED ($F(1, 14) = 9.04$, $p = .009$, partial $\eta^2 = .39$) the animals taking longer to respond on trials when they were incorrect (m = 2379.54 ms, SE = 118.41 ms) than on trials when they were correct (m = 2231.66 ms, SE = 114.25 ms). There was also a main effect of Pair Number ($F(2, 28) = 8.87$, $p = .001$, partial $\eta^2 = .39$) with significantly longer RL on Pair 1 (m = 2404.83 ms, SE = 146.70 ms) than Pair 3 (m = 2221.67 ms, SE = 100.17 ms). There was a significant main effect of Test Type ($F(1, 14) = 7.06$, $p = .02$, partial $\eta^2 = .34$), with RL being significantly longer on CRs (m = 2357.62 ms, SE = 114.40 ms) than on CDs (m = 2253.59 ms, SE = 109.67 ms). There were no main effects of sex ($F(1, 14) = .024$, $p =$

.88, partial η^2 = .002); however a marginal sex X Outcome interaction (F(2, 28) = 3.26, p = .09, partial η^2 = .19) males were significantly slower on trials when they were incorrect (m = 2426.28 ms, SE = 181.89 ms) than when they were correct (m = 2150.73 ms, SE = 175.50 ms) while female RL was unaffected by the outcome of the trial (Incorrect: m = 2332.80 ms, SE = 152.11 ms; Correct: m = 2312.60 ms, SE = 146.77 ms). Sex did not significant interact with Pair Number (F(2,28) = 2.03 p = .15, partial η^2 = .13), or with Test Type (F(1, 14) = .02, p = .90, partial η^2 = .001). A complex sex X outcome X Test Type X Pair Number interaction did emerge (F(2, 28) = 3.27, p = .05, partial η^2 =.19) that suggests males had longer RL on CD3 (Extradimensional Shift) when they were incorrect on the trial (Incorrect: m = 2352.27 ms, SE = 194.94 ms; Correct: m = 1889.55 ms, SE = 160.32).

Non-Responses

There was a significant main effect of Test Type (F(1, 14) = 9.45, p = .008, partial η^2 = .40) indicating monkeys refused more trials on CRs (m = 356.51, SE = 68.25) than on CDs (m = 228.33, SE = 52.76). There was also a significant effect of Pair Number (F(2, 28) = 6.47, p = .005 partial η^2 =.32) with animals refusing more trials on Pair 3 (m = 357.29, SE = 68.37) than on Pair 1 (m = 229.99, SE = 50.73). Sex had no effect on non-responses (p = .72) and did not interact with Test Type (p = .12) or with Pair Number (p = .13).

Discussion

We tested female and male marmosets on two tasks: Simple Reversal Learning a measure of cognitive flexibility, and the ID/ED, a measure of attentional set shifting. We found that females performed more poorly than males on reversal trials of Simple Reversal Learning, especially for Pair 2 and Pair 3, which used stimuli (two shapes or two lines) that were more difficult to differentiate than those of Pair 1 (shape vs. line). Consistent with these findings, females also performed more poorly on the reversal portions of the ID/ED, particularly on Pair 1 and Pair 2 reversal (CR1 and CR2, differences on CR2 significant, differences on CR1 marginally significant) which confronted them with a new pair a reversal of reinforcement contingencies but no change in attentional set (ID reversal). However, they did not have more difficulty than males on the ID or ED shifts per se. These findings point to a specific deficit of females in reversal learning, in the absence of a deficit in attentional set shifting.

Analyses of the latencies showed no sex difference in Reversal Learning but a complex sex X Outcome X Test Type X Pair Number interaction for the ID/ED indicates that males had significantly longer response latencies on ED on trials where they chose incorrectly. With regards to refusals, there were no sex differences in number of trials on which the animals refused to perform, which makes differences in motivation for each task an unlikely explanation for performance differences.

Sex Differences in Reversal Performance

We found a significant male advantage on Simple Reversals Learning, with males needing fewer trials to reach learning criterion on the reversal trials, particularly on the more difficult stimulus pairs, but not the initial discrimination trials, no matter the

difficulty of the pair. This finding is in agreement with human literature, which finds a male advantage in reversal learning in both children (Overman, 2004) and in adults (Evans & Hampson, 2015). Interestingly, there were no sex differences in performance on the first pair of stimuli, which involved the discrimination of two stimuli with clearly different features, a shape and a line of different colors. Only when the stimulus pairs where more difficult to discriminate (two different lines or two different shapes of the same color) did sex differences emerge. This suggests that sex differences in reversal learning are sensitive to the difficulty of the trial, with the male advantage emerging only when the reinforcement contingencies involve discriminating among more similar shape features.

These findings are contrary to Takemoto et al. who failed to find sex differences in marmosets on a similar reversal learning task (Takemoto et al., 2015). There are several differences between the two studies that are worth note. First, the animals in the Takemoto study were 1-4 years old, while our study used older animals (aged 4-6 years). However, given that sex differences in reversal learning are found in both child-aged and adult humans (Evans & Hampson, 2015; Overman, 2004), differences in the ages of animals used seems an unlikely explanation for our discrepant results. Because the data from Takemoto and colleagues was pooled from several different experiments, 14 of the 37 animals were tested using 80% as the learning criterion, while the other 13 were tested using a 90% criterion, as we did in our study. It is possible that using a less stringent mastery criterion masks sex differences in reversal performance. Finally, the stimuli used may provide a plausible explanation. Although complex shapes were employed as stimuli, they were characterized by different color patterns and females could have used

color as a guide for their discrimination, rather than shape. As our experiment did not include trials where color was the only discrimination cue, this hypothesis will need to be validated in the future.

Sex differences in ID/ED Performance

Marmosets needed significantly more trials to reach criterion during the ED than for the ID. This finding is in agreement with previous studies, which have asserted that a shift within the same attentional set (ID shift) is acquired more rapidly than a shift to a new attentional set (ED shift) (Dias, Robbins, & Roberts, 1996b; Owen, Roberts, Polkey, Sahakian, & Robbins, 1991). We also found a significant male advantage on the intradimensional reversal (CR2) with males needing significantly fewer trials than females to reach criterion.

There is ample evidence that reversal learning and attentional set shifting are controlled by anatomically discrete brain regions. The OFC has been shown to be important in Reversal learning paradigms (Izquierdo, Brigman, Radke, Rudebeck, & Holmes, 2017, for review). Functional imaging studies in humans have shown increased activation in the OFC during reversal learning paradigms (Cools, Clark, Owen, & Robbins, 2002; Ghahremani, Monterosso, Jentsch, Bilder, & Poldrack, 2010; Nagahama et al., 2001) and studies in NHPs have shown the lesions to the OFC cause disruptions in reversal but not in initial stimulus-reward associations (Alicia Izquierdo, 2004; Machado & Bachevalier, 2007).

Dias et al. compared performance of marmosets with OFC lesions, animals with lesions to the lateral PFC, and sham-lesioned animals. Marmosets with OFC lesions showed impairments in reversal learning, but ED set shifting remained intact, while

animals with lateral PFC lesions showed opposite deficits. A similar pattern has been shown in rodent research, with lesions to the OFC impairing reversal learning but leaving attentional set shifting intact (McAlonan & Brown, 2003). Thus it is possible that our findings of lower female performance on the simple reversals and on the ID reversal are indicative of sex differences within the OFC but not within the lateral PFC. Further, because our prior study suggests an impairing effect of E2 on reversal learning, these sex differences are likely mediated by E2. There is no basis in the literature to speculate about the potential anatomical differences (number of estrogen receptors, relative distribution of estrogen receptors etc.) or functional differences mediating this sex difference.

Sex Differences in Response Latencies

On the ID/ED, we found slower response latencies on incorrect trials in males, particularly on the extradimensional shift, while female response times were unaffected by outcome. This finding is in agreement with previous work from our lab, which found increased response latencies in males but not females on trials where they were incorrect (LaClair & Lacreuse, 2016). There are several potential explanations for these findings. First, longer response latencies could indicate an increased susceptibility to distraction in males, with this increased distractibility leading to an incorrect choice on long latency trials. However, animals were given a short five-second window within which to make their choices, so it seems unlikely that distraction during increased latency itself would be causing the incorrect choice.

Another possible explanation for increased latencies in males on incorrect trials is that males were more sensitive to punishment (withholding of reward), and so exerted

increased inhibitory control on trials where the outcome was less certain to the animal. Rhesus monkeys and, to a lesser extent, capuchins have been shown to increase inhibitory response control when the outcome of a discrimination task is uncertain (Beran, Perdue, & Smith, 2014). Importantly, this increased inhibitory control was only noted in capuchins when the difficulty of the trial was high. This may explain why sex differences in RL only emerged during the extradimensional shift, arguably the most difficult component of the cognitive testing. Importantly, there was no evidence for a difference in motivation between males and females, based on the analysis of refusals. Although refusals increased with task difficulty (i.e, reversals vs. simple discriminations), the increase was similar in males and females.

Altogether, the results point to a deficit specific to reversal learning in females that may be tightly linked to the difficulty in discriminating among stimuli features. This deficit likely involves the OFC and is independent of the ability to perform attentional set shifting (involving the lateral PFC). Because our prior results indicated that estradiol treatment also impairs reversal learning in OVX females (Lacreuse et al, 2014), it is likely that the impairment in gonadally intact females is related to estrogens. Unfortunately, we were not able to measure potential effects of menstrual cyclicity on performance, as learning was confounded with cycle in our experiment. However, it would be of high interest to determine whether higher estrogen levels during the cycle are associated with greater impairments in reversal learning.

CHAPTER 3

SEX DIFFERENCES IN IMAGING OUTCOMES: MAGNETIC RESONANCE SPECTROSCOPY AND RESTING STATE FUNCTIONAL CONNECTIVITY

Experiment 4: Sex Differences in Magnetic Resonance Spectroscopy (MRS)

In vivo magnetic resonance spectroscopy (MRS) is an imaging technique that allows for the noninvasive examination of the biochemical composition of healthy and diseased brain tissues. MRS allows the regional measurement of several metabolites including: N-acetyl aspartate (NAA), myo-Inositol containing compounds (mI), Choline containing compounds (Cho), Glutamate (Glu), Glutamine (Gln), and Phosphocreatine+Creatine (Cr). Cr levels are usually stable and are used as an internal reference value, while the other metabolites are considered as specific biomarkers (Ross & Sachdev, 2004, for review).

NAA, which is synthesized by mitochondria, is considered to be a marker of neuronal density (Simmons, Frondoza, & Coyle, 1991) and viability (Rutgers, Klijn, Kappelle, & van der Grond, 2000). It is reliably decreased in several brain regions in neurodegenerative diseases. mI is considered to be a suitable marker for glial activity, and is elevated in disease states characterized by inflammation (Chang et al., 2002). Cho is a precursor of acetylcholine that is concentrated in phospholipids and is thought to be a marker for membrane turnover. An increase in Cho has been observed in multiple sclerosis, a disease state known to be associated with diffuse neuronal demyelination (Roser et al., 1995).

High field MRS can also detect several neurotransmitters such as Glu, which is the most abundant excitatory neurotransmitter in the brain. With approximately 80% of

neurons in the cortex and hippocampus utilizing Glu as the primary neurotransmitter (Somogyi, Tamás, Lujan, & Buhl, 1998), is known to play a fundamental role in learning and memory (Riedel, Platt, & Micheau, 2003). Glu released by pre-synaptic neurons is rapidly converted to Gln in astrocytes and Gln released from astrocytes is converted back to Glu, as part as a Glu/Gln cycle that is essential to the normal functioning of brain cells (Ramadan, Lin, & Stanwell, 2013). The combination of Glu and Gln concentrations is traditionally referred to as Glx. Alterations in the concentrations of Glu and Gln have been reported in numerous neurological and psychiatric diseases such as schizophrenia (Merritt, Egerton, Kempton, Taylor, & McGuire, 2016) and major depressive disorder (Horn et al., 2010) and alterations in Glu in both disorders have been linked with cognitive dysfunction (Taylor, Neufeld, et al., 2015).

While ^{1}H MRS is a useful neuroimaging method to identify neurochemical changes associated with a variety of disorders, it can also serve as a tool to investigate potential biomarkers of cognitive performance in the healthy brain (Patel et al., 2014; Ross & Sachdev, 2004). For example, NAA in cortical tissues has been found to correlate with selective aspects of cognitive performance in non-clinical women and men (Patel et al., 2014). Glx concentration in the hippocampus has also been shown to predict verbal memory performance in healthy adult males (Wagner et al., 2016) and delayed word list recall in older adult (Nikolova et al., 2017). Task-based alterations in Glu have also been reported, with increases in Glu in the dorsolateral PFC during a working memory task (Woodcock, Anand, Khatib, Diwadkar, & Stanley, 2018) and increased Glu in the anterior cingulate cortex (ACC) during a the Stroop Task, requiring cognitive control (Taylor, Schaefer, et al., 2015).

We suggest that identifying neurochemical correlates of cognitive performance in a well characterized NHP would provide further validation for the use of specific neurometabolites as biomarkers of cognitive processes. We used ^{1}H MRS in the common marmoset to identify these biomarkers. Despite differences in size and evolutionary distance, many neural features are well conserved between the marmoset and the human brain (Chaplin, Yu, Soares, Gattass, & Rosa, 2013). ^{1}H MRS has been used successfully in this species to measure NAA/creatine ratio in the hypothalamus after MDMA exposure (Meyer, Brevard, Piper, Ali, & Ferris, 2006) and changes in NAA, Cho, and Ino after Modafinil treatment in a 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's Disease model (van Vlieta et al., 2008b). However, none of these studies included cognitive measures so it remains to be determined how metabolite concentrations may predict cognitive performance in marmoset and whether sex modulates this relationship.

Here we focused on cognitive flexibility as assessed by the simple reversal learning task, depending on the OFC (Dias et al., 1996a). Performance on this task is affected by a number of neurotransmitters, including serotonin, dopamine (DA), and Glu (Izquierdo et al., 2017). We predicted that performance on reversal learning would depend on Glu (or Glx) concentrations in the PFC, based on findings that dizocilpine-induced glutamate receptor (NMDA) blockade results in reversal learning impairments in the marmoset (Harder et al., 1998). We did not expect performance to be related to other neurometabolites, as we tested healthy animals younger than 8 years old, when signs of aging begin to appear in marmosets. With regards to sex differences, previous studies in humans have reported region-specific sex differences in a number of metabolites,

including Glu and Gln (Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013). However, our sample was too small to generate strong hypotheses with regards to sex differences.

Subjects

Fifteen marmosets (8 females, mean age = 4.74 years, SD = 0.74; 7 males, mean age = 4.81 years, SD = 0.45) with cognitive data were scanned. The MRS scan occurred within 3 months of the onset of the cognitive test.

Cognitive Testing

A detailed description of the cognitive testing procedures can be found in Chapter II: Experiment 1C.

MRS

The monkeys were scanned at the Center for Comparative Neuroimaging (CCNI) at UMass Medical School (UMMS), Worcester, MA. We briefly anesthetized the monkeys with ketamine (10 mg/kg, IM) to facilitate their positioning in the imaging apparatus. Animals were placed into a sleeveless jacket (Lomir Biomedical, Inc), and earplugs were inserted for noise protection. A plastic semi-cylindrical cover made of LEXAN polycarbonate was attached to the back of the marmoset's jacket using plastic zip ties, as described in Belcher et al. (2013). The marmoset was then placed in a prone position on an MR bed, which consisted of a cylindric tube of inner diameter 111 mm. The cover was secured to the bed with nylon thumb screws and the bed was inserted into the scanner. During the experiment animals were ventilated with isoflurane (2-4%) via a

face cone. Respiratory changes were monitored using a pressure pad (Biopac systems, INc.) placed under the marmoset's body during placement in the MR bed.

Imaging was carried out on a high-field MRI system using a system which incorporated a 4.7T/40cm horizontal magnet (Oxford, UK), equipped with 450 mT/m magnetic field gradients and a 20-G/cm magnetic field gradient insert (inner diameter = 11.5 cm; Bruker, Germany) with a digital interface to Bruker console, run by Paravision 6. Water suppressed ^{1}H MRS data were acquired using a Bruker volume headcoil and the PRESS localization sequence (repetition time = 2500 ms; echo time = 16 ms; averages = 128). Based on previous literature finding an association between Glu in the PFC and reversal performance (Harder et al., 1998), a 3 mm x 3 mm x 3 mm voxel was positioned in the PFC guided by gradient echo localizer images (Fig. 3.1). Un-suppressed water data were acquired for quantification purposes (repetition time = 2500 ms; echo time = 16 ms; averages = 16). Data were transferred to a Linux workstation and metabolite concentrations, in institutional units, were fit using LCModel (Provencher, 1993). A representative marmoset spectrum is shown in Figure 3.2.

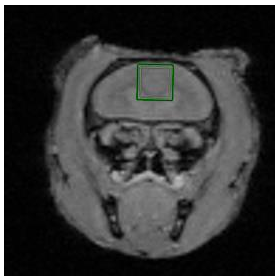

Fig. 3.1. Voxel placement within the PFC

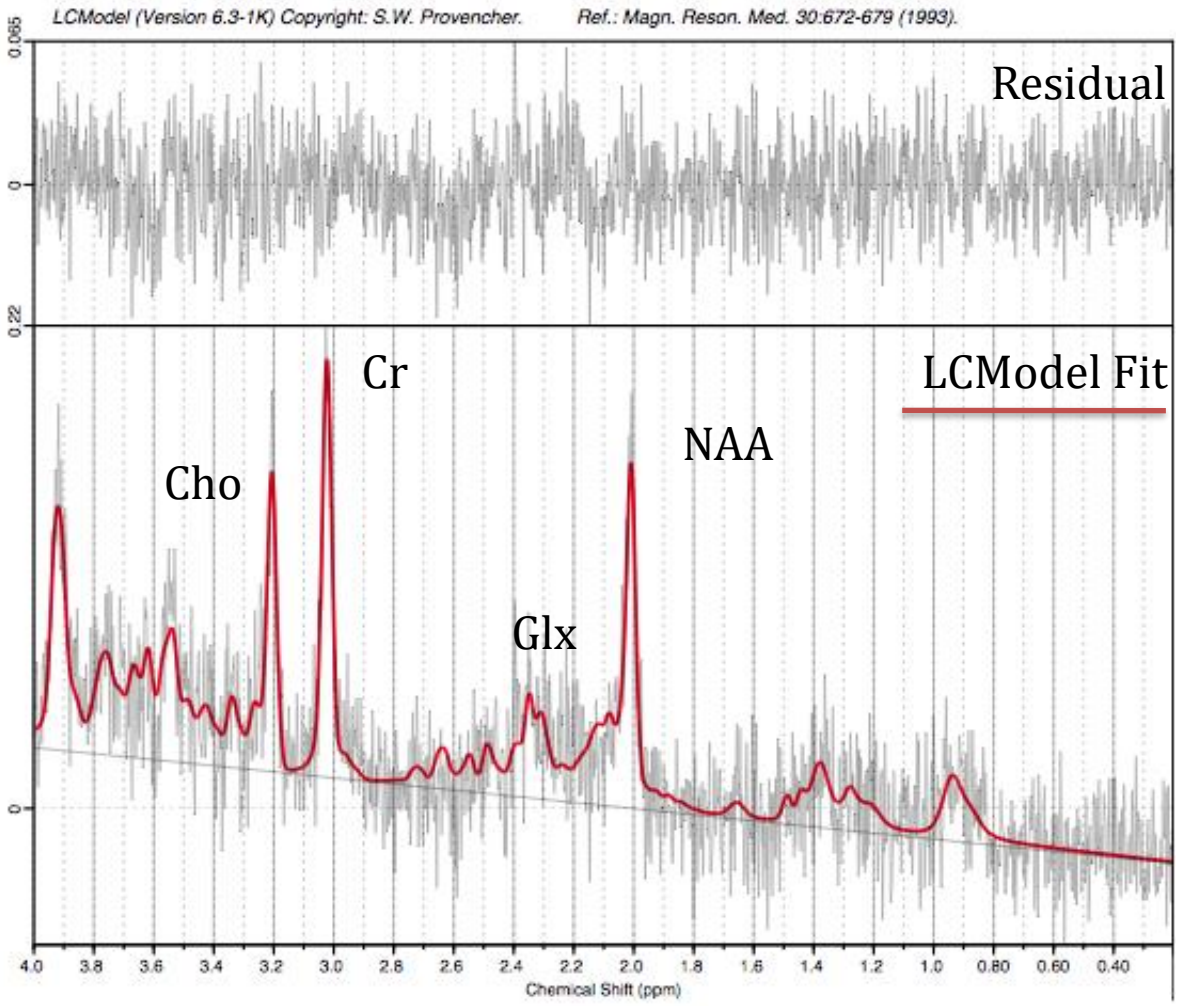

Fig. 3.2. Representative marmoset MRS spectrum

Statistical Analysis

The Mann-Whitney U-test was used to determine whether metabolite concentrations differed as a function of sex. To examine associations between reversal learning performance and metabolite concentrations, a composite of performance, the Reversal Index SR/SD, was computed for each monkey by dividing the mean TTC across the 3 reversals (SR1, SR2, SR3) by the mean TTC on the 3 simple discriminations (SD1, SD2, SD3). This composite score reflected how many more trials the monkey had

needed to perform the reversals relative to the simple discriminations. Associations between SR/SD and metabolite concentrations were assessed using Spearman rank correlations.

Results

Metabolite concentrations did not differ significantly between males and females (Table 3.1). The Spearman's rho correlation coefficient was negatively correlated with Glx (ρ = - 0.643, p = .01, Fig. 3.3) but no other metabolite. This indicated that monkeys who acquired the reversals more quickly (i.e, lower SR/SD ratio) had higher Glx levels. As can be seen in Fig. 3.3, this correlation was driven by the males. Indeed, when only males were considered, a large correlation between SR/SD and Glx concentration (ρ = - 0.821, p = .023) was revealed, whereas it was not significant among females (ρ = - 0.476, p = .23). No other metabolite correlated significantly with SR/SD in either males or females.

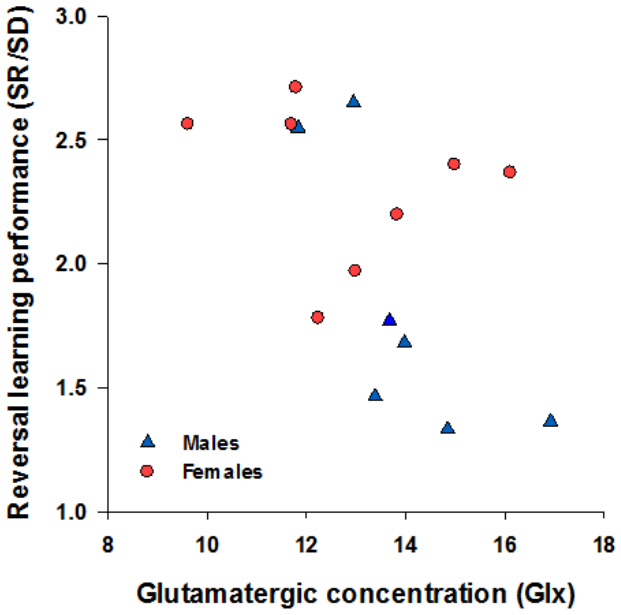

Fig. 3.3 Scatterplot of the relationship between reversal index and Glx in females and males

Monkeys	Metabolite concentrations in PFC														Reversal Learning performance
	Glx	CRLB	Gln	CRLB	Glu	CRLB	mI	CRLB	tCho	CRLB	tNAA	CRLB	tCr	CRLB	SR/SD
Males															
1	12.94	10	4.02	25	8.92	12	6.70	8	1.87	6	6.81	6	8.15	4	2.65
2	13.67	10	3.60	64	10.07	12	9.13	7	2.03	6	7.08	6	8.28	4	1.77
3	13.97	9	3.43	62	10.54	11	7.27	8	1.93	6	8.22	5	8.93	4	1.68
4	11.83	11	3.16	62	8.68	16	7.31	9	1.70	8	7.26	6	6.85	5	2.55
5	16.92	9	5.74	24	11.18	11	7.69	8	1.99	6	8.11	5	8.55	4	1.36
6	14.84	9	6.00	19	8.84	12	7.94	7	2.43	5	7.75	5	8.83	3	1.33
7	13.38	9	3.87	25	9.51	11	5.84	9	1.63	6	8.68	5	8.44	4	1.46
Females															
1	11.79	11	4.04	25	7.76	13	7.87	7	2.39	5	7.65	5	8.32	4	2.71
2	9.61	14	3.35	35	6.26	18	7.48	8	2.26	5	8.07	5	8.66	4	2.56
3	12.99	10	3.66	33	9.33	13	8.20	7	1.92	6	9.02	5	8.12	4	1.97
4	14.98	11	4.43	42	10.55	15	7.33	9	1.76	8	7.57	7	8.44	5	2.40
5	11.70	11	3.86	28	7.84	14	8.42	7	1.93	5	8.47	5	8.79	4	2.56
6	16.11	9	5.54	25	10.57	11	7.64	7	1.99	6	7.14	6	9.22	4	2.37
7	12.24	11	5.19	22	7.05	15	7.83	7	2.02	5	6.55	6	7.81	4	1.78
8	13.83	11	5.05	27	8.78	14	7.30	9	1.39	9	8.77	5	7.88	5	2.20

Table 3.1. PFC Metabolite concentrations, Cramér-Rao Lower Bounds (CRLB) and Reversal Learning performance (SR/SD)

Discussion

This study in marmosets examined potential associations between cognitive flexibility, as assessed by Simple Reversal Learning, and metabolite concentrations in the PFC, as measured by ^{1}H MRS. We found a large correlation (r = - 0.64) between cognitive flexibility and Glx concentrations in the PFC, indicating that better performance on Reversal Learning (lower SR/SD ratio) was associated with higher Glx concentrations in this region. Interestingly, the relationship between Glx and reversal learning was driven by the males, who also outperformed females on the reversals.

The MRS Glx signal reflects the combined concentrations of both Glu and Gln in brain cells located within the voxel of interest. Glu and Gln are tightly coupled; after release, excessive Glu is taken up by astrocytes and converted to Gln by Glu synthetase (Magistretti, 2011). After conversion, Glu is released from astrocytes and taken up by presynaptic neurons, where it is converted back into Glu by mitochondrial glutaminase and stored for future release. This highly dynamic Glu/Gln cycle is critical for the healthy functioning of neurons.

Several studies support a role for Glx in cognitive processes. In a recent functional MRS study, an increase in dorsolateral PFC Glx concentration was found when participants were completing a Letter 2-back working memory task, compared with passive visual fixation (Woodcock et al., 2018), suggesting greater Glu/Gln cycling or increased synaptic glutamate release in response to cognitive task demands. Other studies found that Glx predicted performance on a verbal memory task in both young men (Wagner et al., 2016) and older adults (Nikolova et al., 2017). Our study extends these results for the first time in an NHP, by showing an association between PFC Glx and

cognitive flexibility. The results suggest that greater glutamate availability is associated with better reversal learning performance, consistent with the beneficial role of glutamate in reversal learning (Harder et al., 1998; Izquierdo et al., 2017).

Interestingly, the relationship between Glx and reversal learning was driven by the males. These results suggest that males' reversal learning performance may be more dependent on glutamate than that of females. Previous studies in rodents have established that the glutamatergic system is highly sensitive to sex hormones (Barth, Villringer, & Sacher, 2015) and a few MRS studies in humans also report changes in Glu or Glx as a function of changes in sex steroids. For example, lower levels of Glu/Cr in the medial PFC were found in the luteal compared to the follicular phase in women (Batra et al., 2008) while the use of anabolic steroids was associated with an increase in Glx levels in the ACC in men (Kaufman et al., 2015).

One limitation of our study is that monkeys were anesthetized with ketamine, an NMDA antagonist, which, at anesthetic doses, has been shown to decrease extracellular glutamate levels in the rodent PFC, as assessed by microdialysis (Moghaddam, Adams, Verma, & Daly, 1997). However, with the exception of one study in awake animals (Meyer et al., 2006), MRS studies of the marmoset brain have exclusively used anesthetized preparations ('t Hart et al., 2004; van Vlieta et al., 2008a). Although anesthesia-induced Glu decreases are not likely to be detected with MRS (Chowdhury et al., 2012), it will be important to confirm our findings in awake marmosets to rule out a potential effect of ketamine on Glx. Future studies should utilize ultra-high magnetic fields MR scanners (7 T or higher) to achieve greater voxel specificity within the PFC, to provide an examination of Glx levels within the orbitofrontal cortex, a brain region

known to be involved in reversal learning performance (Dias et al., 1996a). Additionally, animals should be scanned at a time more closely tied to task acquisition.

Despite these caveats, our study shows that PFC Glx is a reliable predictor of reversal learning ability in marmosets, and particularly so in males, who acquired the reversals faster than females. Altogether, these findings suggest that MRS may be a useful tool to detect biochemical markers of cognitive function in healthy NHPs and that biological sex modulates the relationship between specific neurometabolites and cognitive function.

Experiment 5: Sex Differences in Resting State Functional Connectivity (rsFC)

Resting state functional connectivity (rsFC) exploits the BOLD signal to characterize temporally correlated fluctuations in neuronal activity when subjects are at rest, providing a window into network organization in the brain. Resting state networks have been shown to overlap with networks found during fMRI tasks (B. Biswal, Yetkin, Haughton, & Hyde, 1995; Smith et al., 2004) and with structural connectivity measured through diffusion tensor imaging (Van Den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009) Examination of network level neural functioning is important, as coordinated activations and deactivations of brain regions have been shown to be vital in healthy brain function (Fox & Raichle, 2007; Friston, 2011). Indeed, resting state networks have been shown to be altered in patients with major depressive disorder (Zhang et al., 2017), attention deficit/hyperactivity disorder (Zhao et al., 2017), and schizophrenia (Yang et al., 2014) adding to the evidence that rsFC patterns are important indicators of healthy neuronal functioning.

In addition to alterations in rsFC found in individuals with mental illness, variations in rsFC have also been associated with differences in cognitive performance in non-clinical populations. The Default Mode Network (DMN), consisting of the ventral medial PFC, the dorsal medial PFC, and the posterior cingulate cortex, is one of the most thoroughly studied resting state networks (Raichle, 2015). Strength of resting state connectivity within the DMN has been shown to positively correlate with working memory performance (Sala-Llonch et al., 2012). Working memory performance has also been shown to be positively correlated with connectivity between the medial frontal gyrus (MedFG) and the dorsolateral PFC (Sala-Llonch et al., 2012) and between the MedFG and the inferior/superior parietal lobules (Zou et al., 2013).

Sex differences have also been found in rsFC networks (B. B. Biswal et al., 2010). Women have been shown to have greater connectivity within the DMN (Allen et al., 2011). Another study found increased connectivity in frontal and temporal networks in women and increased connectivity in parietal and occipital networks in men (Filippi et al., 2013). Finally, there is also evidence for increased network efficiency in the right hemisphere for males and the left hemisphere in females, congruent with male advantage on spatial tasks and female advantage on language-based tasks commonly found in the literature (Tian et al., 2011). However, direct investigations of the effects of sex differences in rsFC on cognitive performance are sparse. Not all studies agree on the existence of sex differences in rs networks and some argue that sex need not be included as a variable in rs studies (see Weissman-Fogel, Moayedi, Taylor, Pope, & Davis, 2010).

The marmoset is a useful tool for understanding the complex relationship between sex difference in cognition and rsFC. Marmosets can be easily trained to undergo

conscious fMRI scans without the use of any sedative agents (Silva et al., 2011). A recent study by Belcher and colleagues found 12 resting state networks in marmosets that are similar to those found in humans, including 8 sensory networks and 4 "higher-order" cognitive networks (Belcher et al., 2013). However, potential sex differences in rs networks have not yet been examined in marmosets. In this experiment, the marmoset was used to examine sex differences in rsFC and understanding how functional connectivity might relate to cognitive performance in each sex.

Subjects

Eighteen animals with cognitive data (9 female, mean age = 6.12 years, SD = 0.73; 9 male, mean age = 5.88, SD = 0.57 years) were imaged for this study.

General Procedure

We used a state of the art technique, developed by Afonso Silva (Silva et al., 2011), which allowed us to image awake marmosets without the use of anesthetic. Each animal wore a sleeveless jacket (Lomir Biomedical, Inc), which attached to a semi-cylindrical plastic cover made of Lexan, restricting anterior or posterior movement but allowing the animal to move its arms, legs, and tail freely. The plastic cover was attached to the back of the marmoset's jacket using plastic cable ties. The monkey laid in a prone, sphinx position, in the MRI bed, which consisted of a 111-mm cylindrical tube (Fig. 3.4) The cover was secured to the bed by screwing nylon thumb screws into the bars on the bed. Each marmoset wore an individualized helmet adapted to their skull to support the head and prevent movement while providing comfort.

Acclimation

Prior to imaging sessions, animals were acclimated to the bed restraint device, noise related to imaging and the helmet.

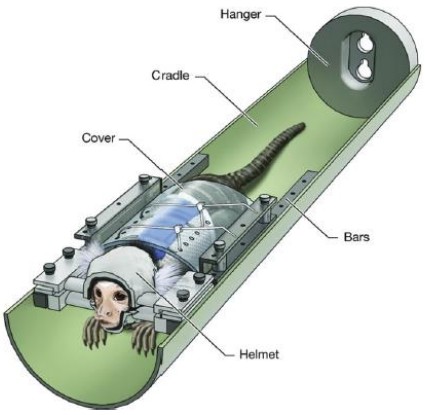

Fig. 3.4 Drawing of marmoset in MRI bed

Bed Restraint (Phase 1): Monkeys were placed in the jacket and attached to the restraint device and then placed into a mock MRI tube in a room with lights dimmed. They were rated on their level of distress using a 1-8 rating scale (see Appendix C for behavioral scoring system). Animals were exposed to the simulator for 15 minutes on their first session. If animals' distress was rated a 3 or below, their acclimation time was increased by 15-minute increments per session until they reach 90 minutes.

MRI Sounds (Phase 2): Animals were restrained in a darkened room as in phase 1, but in phase 2 they were also exposed to (80dB) MRI scanner sounds. Because our first two animals did not show any signs of increased distress to the introduction of the MRI sounds, acclimation for procedure for this step was run as follows:

Day 1 of Phase 2: Marmosets were assessed using the numbered assessment scale every 15 minutes. If the animal was scored a 3 or lower, acclimation continued for another 15 minutes, until the animal's agitation increased or 45 minutes had elapsed.

Day 2 of Phase 2: Marmosets were assessed every 15 minutes as during day 1. Acclimation continued unless agitation scores increased, until 90 minutes had elapsed.

Helmet (Phase 3): Animals were restrained as they were in previous phases, and then

fitted with their customs helmets, which attached to the MRI bed. Because some marmoset distress scores increased when the helmet was introduced, we used the slower increases by 15 minutes per acclimation session as were used in Phase 1. The entire acclimation procedure took between 4-6 weeks for our animals, with acclimation occurring 4-5 days a week.

fMRI data acquisition

The monkeys were scanned at the CCNI at UMMS. Upon arrival, animals were allowed to acclimate for 1 hour prior to scanning. Marmosets were placed in jackets and arranged in the MR bed as described in the acclimation section above and the bed was then inserted into the scanner. Imaging was carried out on a high-field MRI system. The system incorporated a 4.7T/40cm horizontal magnet (Oxford, UK) equipped with 450 mT/m magnetic field gradients and a 20-G/cm magnetic field gradient insert (inner diameter = 11.5 cm; Bruker, Germany) with a digital interface to Bruker console, run by Paravision 6. A surface coil (inter-diameter 2.3 cm) was used for brain imaging. Field map measurements allowed the estimation of the magnetic field inhomogeneity and shimming. For each marmoset, anatomical images were obtained using rapid acquisition relaxation enhanced (T2 Turbo RARE) sequence with TR (relaxation time)=2892.968 ms, RARE factor=8, TE (echo time)=36 ms, resolution matrix=256×256, FOV (field of view)=45 mm×45 mm, slice number=25 slice thickness=1.1 mm. Representative anatomical images are presented in Figures 3.5a-d. Functional images were acquired using echo-planar imaging (EPI) with the same FOV and slice thickness, TR=1691.038ms, TE=26.523 ms, flip angle=90°, and resolution matrix=128×128, for 22.5 min (400 repetitions).

Data pre-processing

Data pre-processing and analysis was performed by CCNI personnel at UMMS. The brain was isolated in the anatomical images using the Brain Extraction Tool (BET; Smith, 2002) in the FMRIB's Software Library (FSL; Smith et al., 2004). Functional images were pre-processed using FMRI Expert Analysis Tool (FEAT 6.00; Woolrich et al., 2008) in FSL. Steps included motion correction applied using MCFLIRT, removal of non-brain tissues based on the anatomical mask, spatial smoothing using at 1 mm full-width-at-half-maximum Gaussian kernel, and high-pass temporal filter cutoff at 150 seconds. EPI images from each marmoset was co-aligned with the anatomical scan acquired during the same scan. Anatomical scans were spatially aligned to high-resolution template marmoset brain (Hikishima et al., 2011), with the transformation for each marmoset likewise applied for the functional scan.

Resting-state fMRI data were analyzed using group-level independent component analysis (gICA) using Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) in FSL (Belcher et al., 2013; Hutchison et al., 2011; Mantini, Corbetta, Romani, Orban, & Vanduffel, 2013). ICA is a powerful, data-driven technique used in fMRI to identify functional networks, those voxels that co-activate temporally, without the need to specify explicit time series a *priori* (Beckmann & Smith, 2005; V D Calhoun, Adali, Pearlson, & Pekar, 2001; Vince D Calhoun & Adali, 2006; Vince D Calhoun, Adali, & Pekar, 2004; McKeown et al., 1998). It allows for the separation of linearly mixed sources. We considered models specified by 25, 30, or 35 components, as prior studies report an optimal number for nonhuman primates between

20 and 30 components, beyond which there is typically excessive fractionation of the resting state networks (Belcher et al., 2013; Hutchison et al., 2011; Mantini et al., 2013).

Each separate so-called "component" obtained represents a source of signal, with a proportion of components reflecting brain networks, and others artefactual noise (cerebrospinal fluid, physiological or scanner noise). Extracted component maps were visualized in FSLeyes. Identification of marmoset anatomy and brain regional definitions is based on a marmoset brain atlas (Paxinos, Watson, Petrides, Rosa, & Tokuno, 2012). All results are displayed as overlays onto the anatomical data. Statistical testing for associations between the network components and factors of interest were obtained upon dual regression and FEAT, with a primary focus on an interaction between the reversal index (SRSD and sex), and exploration of other behavioral measures.

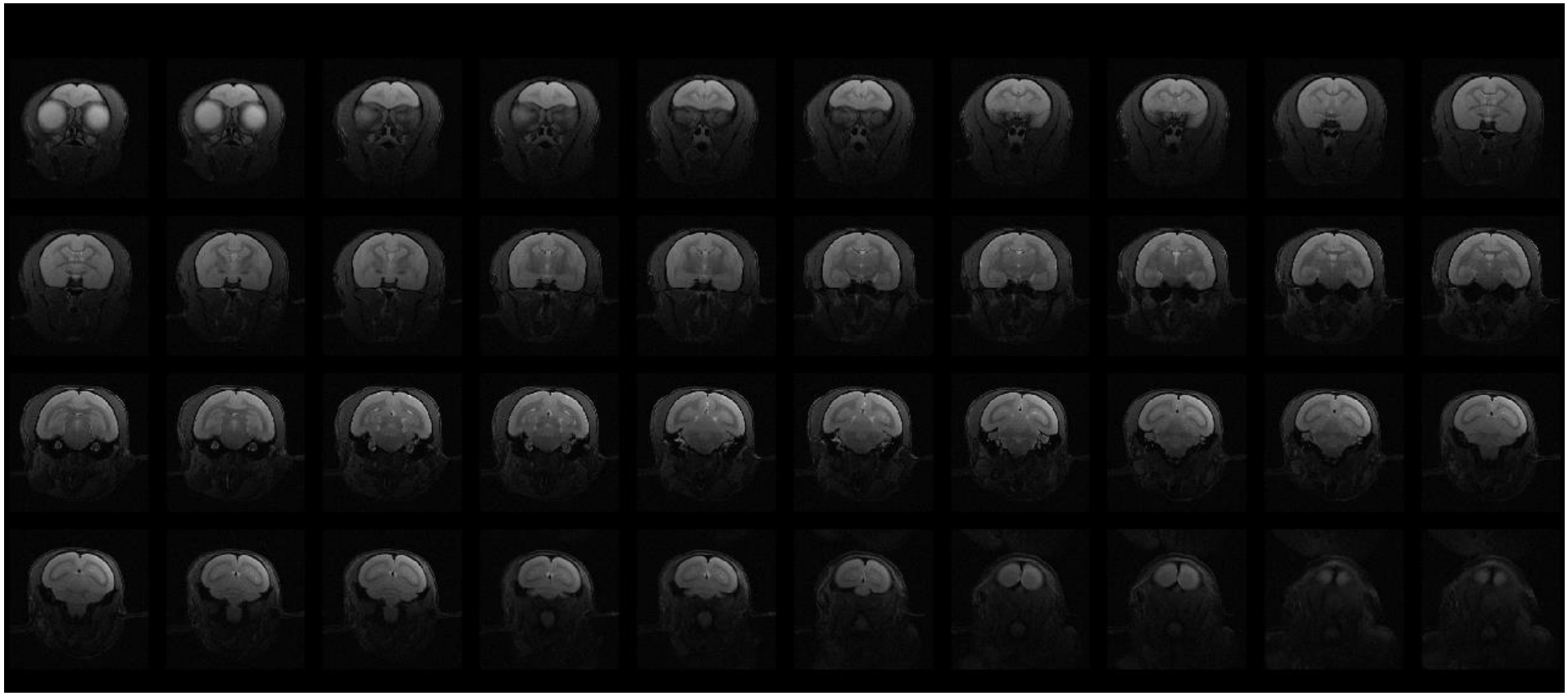

Fig. 3.5a Representative anatomical image

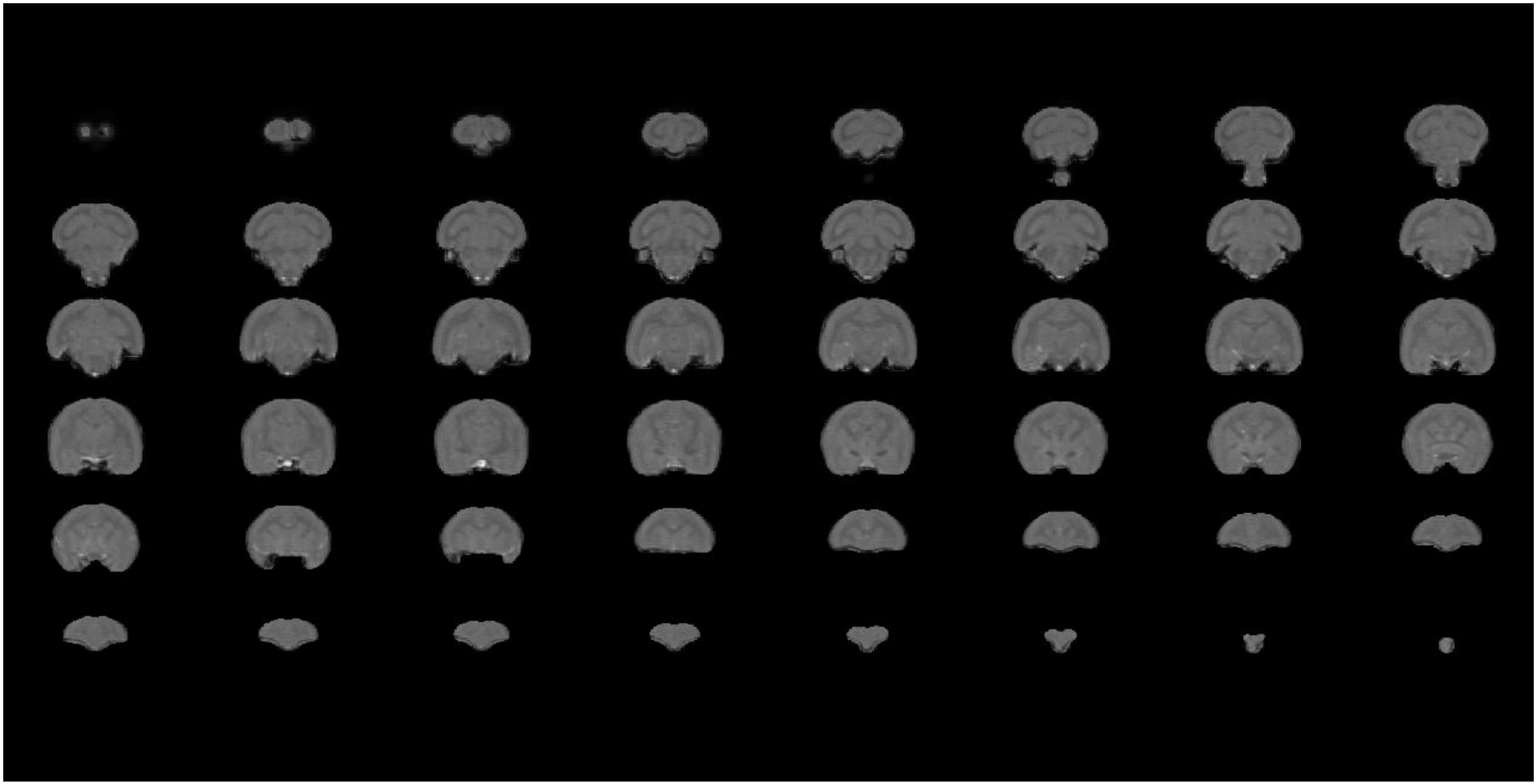

Fig. 3.5b Representative anatomical image registered to template (coronal)

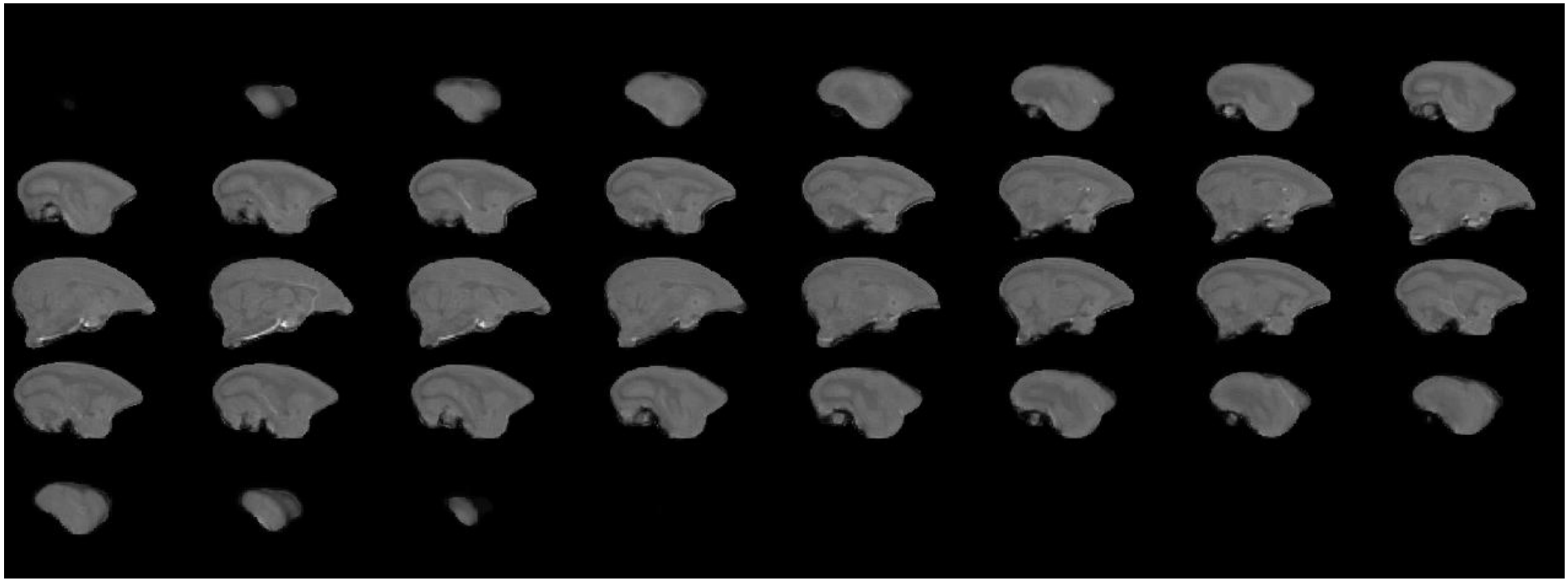

Fig. 3.5c Representative anatomical image registered to template (sagittal)

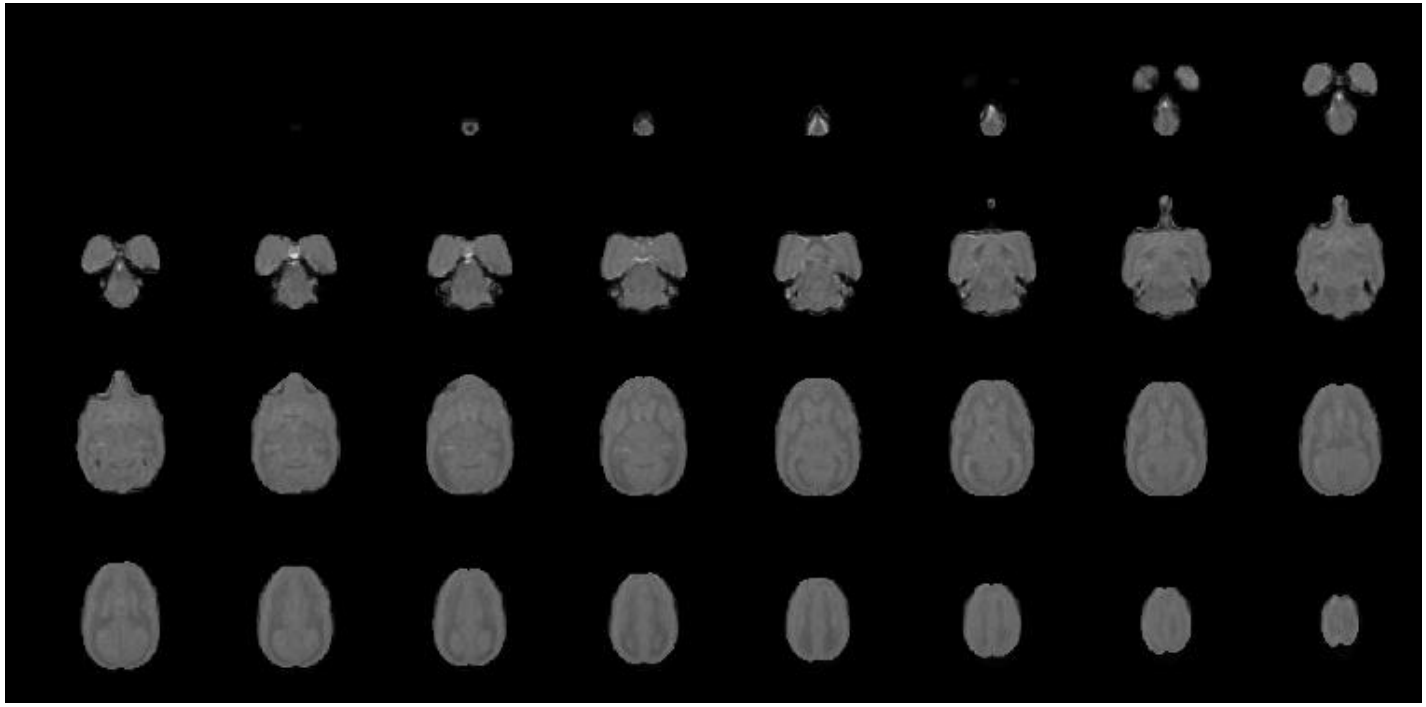

Fig. 3.5d Representative anatomical image registered to template (horizontal)

Results

The model specified by 25 components performed best. Models using 30 and 35 components resulted in excessive fractionation, including splitting hemispheres and breaking down known networks into subnetworks. Note that the greatest amount of variance is explained according to descending component order. Of the 25 components produced using MELODIC group ICA, 9 were clearly related to movement (or other physiological/scanner artefacts; 00-02, 05-06, 08-09, 12, 21). Of the remaining components, 12 comprised some identifiable features of expected networks. Components 03, 04, 06, 15, 19, 20 identified as aspects of visual networks (04 potentially comprising aspects of dorsal attention network). Basal ganglia were represented in network 10, a portion of salience network in network 13, dorsomedial somatomotor network in network 14. Finally, frontal regions are represented in networks distinguished in components split 23-24 (Figure 3.6), and anterior default-mode/frontal pole in 11.

Analyses of sex differences revealed greater connectivity in females for prefrontal network 24 ($p = 0.0164$), basal ganglia network 10 ($p = 0.0178$) and visual network 19 ($p = 0.0488$).

Examination of the sex and reversal index revealed a trend for an interaction, specifically for the slope of a prefrontal network with reversal index (SR/SD), for which Female > Male, $p = 0.0534$ (network 24, Figure 3.7). Based on the behavioral results identifying an interaction between these two variables, follow-up analyses were conducted. To interpret this observation, the slopes for each sex were tested, i.e. the association of resting connectivity with SR/SD separately for each sex. The slope of a prefrontal network #24 with SRSD in males is negative ($p = 0.0412$), whereas that for

females is not different from zero ($p > .4$). suggesting better performance on reversal learning in male monkeys (but not females) that had stronger connectivity with areas outside of the network.

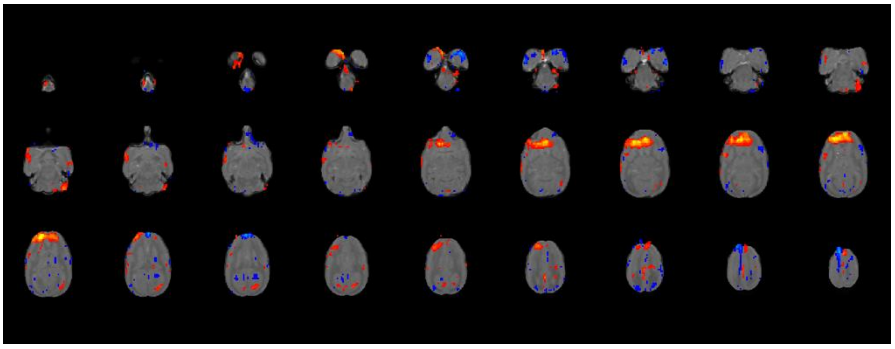

Fig. 3.6 Illustration of component #24 (prefrontal network)

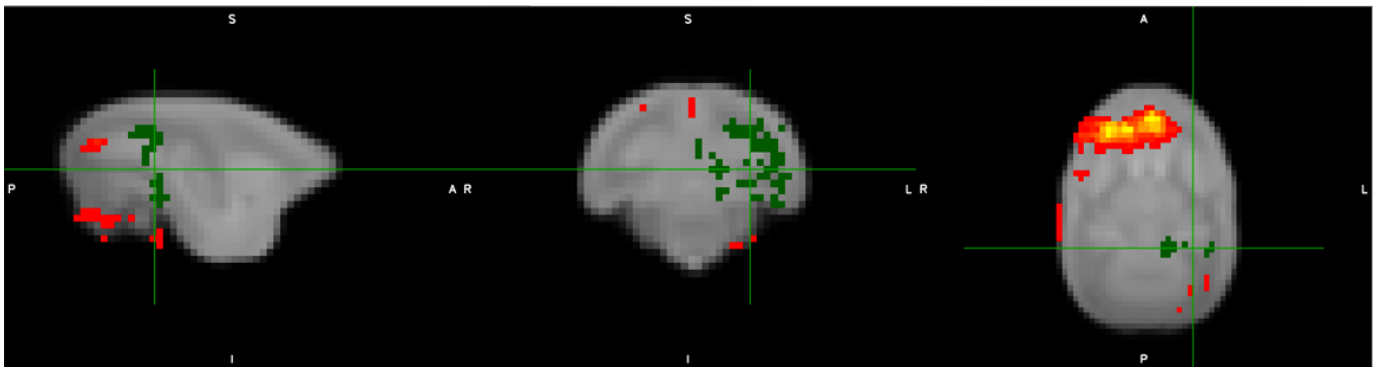

Fig. 3.7 Illustration of component #24 with overlaid representation of sex and SRSD interaction (for visualization purposes, thresholded at $p = 0.055$).

Additional analyses for separate variables of interest (i.e. no adjustment for sex or otherwise) revealed that SRSD and total trials to criterion (TTC) show significant negative association in the same network (#11, SRSD, $p = 0.0048$; TTC, $p = 0.0054$), but

in different regions. Finally, there was no association of any network connectivity with the Non-responses.

Discussion

It is important to first note that numerous studies have found that rsFC closely relates to networks observed during task evoked fMRI (Biswal et al., 1995; Fox & Raichle, 2007). Regions that co-activate with a seed region in task-evoked fMRI tend to be positively correlated with the seed region at rest. We can thus consider rsFC to be reflecting natural propensity of network function.

In agreement with previous rsFC studies in marmosets (Belcher et al, 2013), we found representations of multiple resting state networks found in humans, including two resting state networks that are also activated in response to particular cognitive demands: the dorsal attention network (network 4), involved in top-down and stimulus driven attention (Fox, Corbetta, Snyder, Vincent, & Raichle, 2006) and the salience network (network 13), responsible for using sensory data regarding the visceral, autonomic, and hedonic value of stimuli to guide behavior (Seeley et al., 2007). Aspects of the dorsomedial somatomotor network (network 14), a rsFC network that has been featured prominently in examinations of both humans and NHPs, was also found represented in our animals (Beckmann & Smith, 2005; Belcher et al., 2016; Hutchison et al., 2011; Mantini et al., 2013). Concordance between human and marmoset resting state networks further strengthens the evidence that the marmoset is a useful model in studies of cognition and resting state networks.

A sex difference emerged in the prefrontal network #24 when sex and cognitive performance, as assessed by the Reversal Index SR/SD, were included in the regression analyses. First, greater connectivity was observed in females in network # 24. In addition, a nearly significant interaction ($p = .0534$) between sex and SR/SD on the slope of this network indicated differences between males and females in the relationship between RSFC and cognition. Subsequent analyses confirmed that for males, the relationship was negative, suggesting better performance on reversal learning in monkeys that had stronger connectivity with areas outside of the network. There was no significant relationship between Prefrontal Network extension and performance in females. Since females performed worse on the reversals, it is possible that network extension provides a neural correlate for male advantage on the Reversal task.

CHAPTER 4

BRAIN/BEHAVIOR RELATIONSHIPS AND GENERAL DISCUSSION

Brain/Behavior Relationships

While it is important to understand the ways that sex impacts motor ability, stress reactivity, and metabolite concentration in the brain, it is also vital to examine how differences in these domains may impact sex differences in cognitive performance. To gain a clearer understanding of the factors that contribute to sex difference in cognitive performance on the Simple Reversal Learning task, we created a multiple regression model which considered the effects of sex, motor ability, cortisol levels, Glx concentration in the PFC, and rsFC within the PFC network (#24) on reversal index.

Subjects

Data from 22 marmosets (11 female) who completed Simple Reversal Learning were used in the regression analysis. The regression analysis removes animals that do not have data for all predictors, so the final number of animals included in the regression was 11 (6 female).

Statistics

The predictors considered for the model were sex, Valley Left score, Valley Right score, Hill Left score, Hill Right score, Mean Hill and Valley score, baseline cortisol (BLCort), change in cortisol from baseline to endpoint separation (EndCortChange), change in cortisol from baseline to the time point in which animals' cortisol levels were highest (MaxChangeCort), Glx concentration, and rsFC within the PFC network (#24). Correlations were run between all predictor values to assess collinearity and between predictor values and reversal index to assess fitness of predictor value in model.

Results

Correlations

Both sex ($r(20) = .64$, $p = .001$) and Glx ($r(13) = -.53$, $p = .04$) were significantly correlated with reversal index. Baseline cortisol ($r(20) = .41$, $p = .06$) was marginally significantly correlated with reversal index. The correlation between rsFC in the PFC network and reversal index was not significant ($r(15) = .24$, $p = .39$); however, because the relationship between rsFC in the PFC and reversal index was already established in our previous experiment, we chose to include this variable in the fitting of the regression model. Because the motor measures, EndCortChange, and MaxCortChange were not significantly correlated with reversal index but were correlated with several of the other predictors, these measures were not included. See Appendix D for full correlation matrix.

Regression

The multiple regression predicted reversal index based on sex, Glx concentration, BLCort, and rsFC in the PFC network (#24). See Table 4.2 for β values for each predictor. The regression equation was significant ($F(4, 6) = 12.87$, $p = .004$) with adjusted R^2 value of .83. Predicted reversal index is equal to 1.341 - .039(Glx) - .002(rsFCPFC) - .019(BLCort) + .924(Sex), when sex is coded as 1 = male, 2 = female. Sex is a significant predictor of reversal index ($p = .001$), rsFC in the PFC is a marginally significant predictor ($p = .12$), BLCort was a marginally significant predictor ($p = .10$), and Glx concentration was not a statistically significant predictor ($p = .28$). However, including Glx concentration in the model improved the predictive value of the model (adjusted R^2 without Glx = .74).

Coefficients							
Model	Unstandardized Coefficients		Standardized Coeffecients			95.0% Confidence Interval for B	
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	1.341	.573		2.341	.058	-.061	2.743
Sex	.924	.146	1.008	6.334	.001	.567	1.281
GLX	-.039	.033	-.169	-1.181	.282	-.121	.042
PFCConnectivity	-.002	.001	-.264	-1.817	.119	-.004	.001
BLCort	-.019	.010	-.276	-1.938	.101	-.044	.005

Table 4.1 Coefficients table for multiple regression

General Discussion

The first objective of this dissertation was to examine sex differences in motor ability, stress reactivity, and cognitive performance in the marmoset. Experiment 1 tested the hypothesis that female marmosets would outperform male marmosets on a task of fine motor control, the Hill and Valley task, which requires animals to use either the contralateral hand and visual field (Valley) or the ipsilateral hand and visual field (Hill) to retrieve treats from a narrow aperture. We found that all animals, independent of hand preference, performed better with the right hand when the ipsilateral hand and visual field (Hill) were required. However, when the contralateral hand and visual field were required, only females showed the right-hand advantage, potentially indicating superior perceptual-motor integration in females.

Experiment 2 examined whether males and females would differ in their response to a social stress paradigm. In the Social Separation Stress test, based on the procedure used by Jeffrey French's lab (French et al., 2012), focal animals are removed from the colony and placed in a new room/cage for 7 hours. Based on previous work in our lab,

we hypothesized that females would show a more robust behavioral and cortisol response to separation. Females showed a significant increase in agitated locomotion, a known behavioral indicator of stress in marmosets, compared to males. Additionally, while both males and females had similar overall cortisol responses to the social stress, females had a significantly greater increase in cortisol at the midpoint (~ 3.5 hours after onset) of separation, compared to males. These data suggest that females were more reactive to the social stressor than males.

When stress reactivity is examined in humans, sex differences in stress response seem to be specific to the type of stressor, with men being more robustly affected by performance/academic stress than women (Liu et al., 2017) and women showing greater stress response to social stress (Stroud et al., 2002). Interestingly, while men show a greater cortisol response during performance/academic stressor, they report no greater levels of anxiety/distress than women (Stroud et al., 2002). This discrepancy between the physiological and psychological expression of stress is reminiscent of our findings in marmosets, where although overall increase in cortisol was similar between the sexes, females showed a more robust behavioral response to the stressor.

While we did not find sex differences in the overall increase in cortisol in response to our social stressor, sex differences in stress vulnerability are well documented, and can likely be attributed in part to close reciprocal interactions between the HPA and the Hypothalamic-Pituitary-Gonadal (HPG) axes (Oyola & Handa, 2017, for review). Treatments with exogenous 17-β estradiol has been shown to increase cortisol, independent of stressor, in gonadally intact female baboons (Giussani et al., 2000) and OVX female rhesus female cynomolgus macaques (Stavisky et al., 2003). In

particular, sex hormones affect HPA axis reactivity, as measured by the release of cortisol or its precursor adrenocorticotrophin hormone (ACTH). For example, exercise-induced stress in women increases ACTH, but only during the mid-luteal stage of the menstrual cycle when estrogens are relatively high (Altemus, Roca, Galliven, Romanos, & Deuster, 2001). In future research, it will be important to measure estradiol levels in females before undergoing social stress, in order to characterize the effect that differences in estradiol levels may have on the behavioral and cortisol response to the stress paradigm.

It is important to note that while women show an increased cortisol response to social stress, the overall increase in cortisol in response to social stressor in our animals was similar between the sexes. This difference is possibly due to the length of exposure to stressor. In the Yale Interpersonal Stressor (YIPS), a common social stress paradigm pioneered by Laura Stroud in which women show a greater increase in cortisol than men, exposure to social stress is usually 5-15 minutes (Stroud et al., 2002; Stroud, Tanofsky-Kraff, Wilfley, & Salovey, 2000). In our experiment, animals were socially isolated for seven hours, a much longer exposure to stress than what is usually used in human experiments. Furthermore, at the midpoint of the stressor, female animals had a significantly greater increase in cortisol than males. This may indicate that males are insensitive to shorter periods of social stress, but show a similar cortisol response to females to extended social stress.

Experiment 3 investigated sex differences in cognitive performance in two different PFC-related tasks: Simple Reversal Learning, a task dependent on the OFC, and ID/ED Set Shifting, a task dependent on the dorsolateral PFC. The Simple Reversal

Learning task required animals to learn to associate one of two test stimuli with reward, and then inhibit the previous response and learn to choose the opposite stimulus when reward/response contingencies were reversed. Based on literature in humans, we hypothesized that females would perform more poorly on the reversal portion of this task than males (Evans & Hampson, 2015; Overman, 2004).

While females and males performed similarly on the initial stimulus discrimination, we found that females needed significantly more trials than males on the reversal trials, and that this effect was specific to pairs in which the stimuli were more difficult to discriminate. This is in concordance with human literature, which finds that sex differences in reversal learning are difficulty dependent, with less difficult reversal tasks failing to show sex difference (Overman, 2004). Importantly, there were no sex differences in the number of trials in which the animal refused to choose a stimulus, which indicates that these results are not based on differences in motivation.

It is possible that increased estradiol levels in females compared with males is responsible for the female disadvantage in reversal learning. Previous work from our lab found that OVX females treated with E2 performed more poorly on reversal learning than OVX females treated with placebo (Lacreuse et al., 2014). The pattern of decreased reversal performance with E2 treatment has also been found in rats (Gibbs, Chipman, Hammond, & Nelson, 2011). It is also possible that age may also play a role with E2 treatment increasing impulsive choice in young and middle aged, but not old rats (Wang, Neese, Korol, & Schantz, 2009). Additionally, work in humans shows that women with a genetic polymorphism that results in increased DA in the PFC show impaired working memory performance when tested during the high E2 portion of the cycle (Jacobs &

D'Esposito, 2011). Unfortunately, in our experiment, rate of learning was confounded with cycle phase and it was not possible to ascertain how changes in E2 levels affected performance overtime in our sample. Future experiments should control for changes in E2 levels, either through the use of OVX animals, or by examining human participants, who can complete a reversal learning task in a single lab session, rather than over the course of weeks or months, as is the case with animals.

The ID/ED Set Shifting task required animals to actively update reversal contingencies as in Simple Reversal Learning; however, the ID/ED had the added complexity of requiring animals to also shift attentional set. We hypothesized that females would continue to perform more poorly than males on reversals, but did not expect sex differences in set shifting. We found that females performed significantly more poorly than males on the reversal for the second pair of stimuli (ID reversal, CR2), in agreement with our results from the Simple Reversal Learning paradigm. As expected, there were no sex differences in performance on the ED set shift (stimulus pair 3, CD3). Contrary to our expectations, we did not find any sex differences in performance on the ED reversal (CR3).

The sex difference in performance from Simple Reversal Learning, with females performing more poorly on reversal learning than males, was maintained on the ID reversal stage (CR2) of the ID/ED. The ID shift and reversal (CD2, CR2) requires animals to discriminate between stimuli within the same dimension salient in the compound discrimination and reversal (CD1, CR1). Because no change in attentional set was required for accurate performance on the ID shift and reversal, the emergence of a

male advantage on the ID reversal reinforces our theory that female performance on the reversal is particularly affected by the difficulty of the discrimination.

Female and male performance was similar on the ED shift (CD3). This result was expected, as there is no evidence of sex differences in attentional set shifting in the existing literature, and this task has been shown to depend on a different PFC region (i.e. dosrsolateral PFC) than reversal learning (i.e. OFC). All animals did take significantly more trials to research criterion on the ED shift compared with the ID shift, a pattern of performance previously reported in marmosets (Roberts et al., 1988), macaques (Weed et al., 1999) and humans (Roberts, 1996). Behavioral homology among these species shows the usefulness of the marmoset as a model for human cognitive performance.

Contrary to our expectations, we did not find any sex differences in performance on the ED reversal (CR3). In humans, performance on the ED shift and the ED reversal are similar in terms of trials and errors to reach learning criterion (Owen et al., 1991). This similarity is taken to mean that the participant is using the information from the extradimensional set shift to guide performance in the extradimensional reversal. Our animals took significantly more trials to learn the extradimensional reversal compared to the extradimensional shift ($p < .001$), similar to performance in rhesus monkeys on this task (Weed et al., 1999). This significant increase in trials to reach criterion on the extradimensional reversal indicates that marmosets may not have incorporated the attentional set shift into their stimulus choice. Rather than choosing between the two stimuli from the relevant dimension, animals may have treated all four stimuli as equally likely to provide reward. Because this difficulty in utilizing the information from the

extradimensional shift, it is possible that any potential sex differences on the reversal were obscured by floor effects.

The second objective of this dissertation was to investigate the neural substrates underlying sex differences in cognitive performance as assessed by MRS (Experiment 4) and rsFC (Experiment 5) in marmosets, and to quantify how these measures impact cognitive performance. Experiment 4 utilized MRS to quantify concentrations of N-Acetyl Asparate, Myo-Inositol containing compounds, Choline containing compounds, Phosphocreatine + creatine, Glutamate (Glu) and Glutamine (Gln) in a 3 mm^3 voxel positioned in the marmoset PFC. Based on previous research showing that antagonism of glutamate receptors impairs reversal performance in marmosets (Harder et al., 1998) we hypothesized that Glu or Gln or Glx (Glu + Gln) concentrations would be correlated with performance on the Reversals task. We used reversal index, computed for each animal by dividing the mean TTC for the reversals by the mean TTC for the initial discriminations for correlation with Glx. Existing data did not allow us to make any strong hypotheses about sex differences in Glx concentration or sex differences in the relationship between Glx and reversal index.

We found that Glx was negatively correlated with reversal index, indicating that greater concentrations of Glx in the PFC were associated with better reversal performance. While there were no sex differences in Glx concentrations, we found that the correlation between Glx and reversal index was driven by males, indeed, when males were removed from the analysis, the relationship between Glx and reversal index was no longer significant for females. This indicates that males' performance may be dependent on synaptic efficiency for utilizing Glu.

In Experiment 5, we used fMRI to measure rsFC networks in the marmosets. Because rsFC is in its infancy, there is a lack of data examining the ways in which sex differences in resting state networks may affect cognitive sex differences. However, multiple studies have found correlations between strength of resting state networks and cognitive performance (Sala-Llonch et al., 2012; Zou et al., 2013), and as such it was hypothesized that stronger connectivity within the PFC would be associated with better reversal learning performance. First, we found greater connectivity within the PFC network in females compared to males. Second, a significant relationship between the strength of recruitment of areas outside the PFC network and cognitive performance was found in males but not in females. This indicated that greater recruitment of brain regions outside the PFC network was associated with better reversal learning performance in males, whereas network extension had no effect on reversal learning performance in females.

These sex differences may be due to the impact of sex steroids on rsFC; however there is lack of research directly investigating connections between sex hormones and resting state connectivity. One recent human study found that plasma testosterone levels were negatively correlated with resting state connectivity in some regions of the frontal cortex including the superior frontal gyrus (Mueller, Wierckx, Jackson, & T'Sjoen, 2016). The authors report that while these effects were significant, the data was taken from a relatively small sample (n = 21 men) and thus should be viewed with caution.

Evidence concerning the effects of the menstrual cycle on resting state networks is mixed. One recent study in humans found that women within the follicular phase of the menstrual cycle had greater connectivity in two networks important for cognitive

performance, the Default Mode Network and Executive Control Network, than women who were in the luteal phase of their cycle (Petersen, Kilpatrick, Goharzad, & Cahill, 2014). However, Hjelmervik and colleagues failed to find an effect of menstrual phase on resting state connectivity in four fronto-parietal networks (left dorsal, ventral, right dorsal, anterior networks) associated with cognitive performance (Hjelmervik, Hausmann, Osnes, Westerhausen, & Specht, 2014). Interestingly, this group did find sex differences, unrelated to the cycle, in the right dorsal and ventral regions, with women showing greater connectivity, particularly in the prefrontal regions. Unfortunately, we do not have cycle data on our females, but future investigations of rsFC should include a measure of menstrual cyclicity, as well as measure of testosterone in males.

Altogether, the findings of the behavioral portion of this dissertation show that sex differences in motor performance, stress reactivity, and cognitive performance in marmosets are similar to those found in humans performing analogous tasks. This similarity strengthens the viability of the marmoset as a model of human cognitive performance. The results of the neuroimaging studies further strengthen the viability of the model, by showing that multiple resting state networks found in humans can also be found in the marmoset, in agreement with other marmoset work (Belcher et al., 2013). Finally, the results from both the MRS and the rsFC studies provide potential biological explanations for sex differences in reversal learning, with both Glx in the PFC and PFC network extension being associated with better performance in reversal learning in males, but not in females.

In conclusion, our data in marmosets stress the importance of taking sex into account for both behavioral and neuroimaging endpoints. Future studies should examine

whether sex differences change with age, as this may have important implications for human health. Indeed, recent human data point to sex differences in age-related cognitive decline, with males showing steeper decline than women as they age (McCarrey, An, Kitner-Triolo, Ferrucci, & Resnick, 2016). Using an animal model like the marmoset, in which rapid aging makes longitudinal studies feasible, may help identify the factors that contribute to differential cognitive decline between the sexes and set the stage for sex-specific treatments for age-related cognitive impairments.

APPENDIX A

MARMOSET BEHAVIORAL ETHOGRAM

Behavior	Definition
Vocalization	Any sound made from mouth, including chirps, whistles, and chittering
Aggress	Grapple with another marmoset, involving biting, clawing, and wrestling, and chasing
Displace	Takeover of position of another animal
Agitated Locomotion	Moves more than one step in a directed plane, exaggerated gait, can be accompanied by piloerection, tail may be extended or arched
Calm Locomotion	Moves more than one step in directed plane, relaxed gait, not agitated locomotion
Inactive Alert	Sitting stationary for more than 3 seconds, animals is awake and actively scanning surroundings
Inactive Rest	Sitting stationary for more than 3 seconds, relaxed facial expression, eyes may be open or closed, visual scanning of environment minimal
Headcock	Turning of the head in inspection of an observer, animal, or object
Genital display	Raise tail to expose genitals
Scentmark	Rub or drag anogenital, suprapubic, or sternal region along substrate, object, or partner
Scratch	Vigorous rubbing of a body part
Tuft-Flick	Rapid back-and-forth movement of ear tufts
Tactile Oral	Sniff, bite, chew, handle, or otherwise manipulate inanimate object, excluding food items and water bottle, for at least 1 sec
Eat	Consumption of food
Drink	Licking or sucking on water bottle
Social Contact	Passive close contact with another marmoset, within an arm’s length, with both animals remaining stationary and in passive contact for at least 3 sec
Sniff/Nuzzle	Orient face against or toward partner, excluding anogenital region
Mount	Climb on partner's back from behind and grip partner around waist and legs; may be accompanied by pelvic thrusting
Social Play	Social interactions involving non-aggressive physical contact with other individuals; high activity
Self Play	Repetitive movements toward objects or fixtures in cage, may include spinning, swinging, and hanging
Social Groom	Use hands and/or mouth to pick through fur and/or mouth of partner, excluding anogenital region
Self Groom	Licking, picking or spreading of one’s own hair or skin
Other	Sneezing, coughing, piloerection or any other behavior not identified

APPENDIX B

IMPUTATION DATA ED REVERSAL

Imputation Number	Animal	CD1	CD2	CD3	CR1	CR2	CR3
0	Nolan	116	346	2227	379	737	.
1	Nolan	116	346	2227	379	737	2799
2	Nolan	116	346	2227	379	737	4106.5
3	Nolan	116	346	2227	379	737	2297.1
4	Nolan	116	346	2227	379	737	3516.1
5	Nolan	116	346	2227	379	737	3741.3
6	Nolan	116	346	2227	379	737	3658
7	Nolan	116	346	2227	379	737	4556.1
8	Nolan	116	346	2227	379	737	2746.5
9	Nolan	116	346	2227	379	737	2498.9
10	Nolan	116	346	2227	379	737	3103.3

APPENDIX C

FMRI ACCLIMATION BEHAVIORAL SCORING

Description of Acclimation Behavior	**Score**
Quiet: marmoset calm and relaxed	**1**
Mostly quiet, agitated only initially	**2**
Mostly quiet, with brief mild agitation	**3**
Quiet after initial struggle, increasingly agitated over time	**4**
Mild agitation for about half of the restraint period	**5**
Moderate agitation during half of the restraint period	**6**
Restless and agitated during most of the restraint period	**7**
Extremely agitated during most of the restraint period	**8**

APPENDIX D

CORRELATION MATRIX FOR POTENTIAL MULITPLE REGRESSION PREDICTORS

Correlations

		Sex	SRSD	GLX	PFCConnectivity	ValleyRH	ValleyLH	HillLH	HillRH	MeanMotor Score	BLCort	EndCort Change	MaxCort Change
Sex	Pearson Correlation	1	.635**	-.284	.433	.484	-.050	.000	-.120	.110	.243	.084	.159
	Sig. (2-tailed)		.001	.304	.107	.068	.855	1.000	.658	.685	.277	.725	.504
	N	22	22	15	15	15	16	16	16	16	22	20	20
SRSD	Pearson Correlation	.635**	1	-.527*	.240	.307	.043	.080	.088	.184	.407	-.002	.076
	Sig. (2-tailed)	.001		.043	.389	.265	.875	.768	.746	.496	.060	.993	.751
	N	22	22	15	15	15	16	16	16	16	22	20	20
GLX	Pearson Correlation	-.284	-.527*	1	-.224	-.752**	-.791**	-.268	-.375	-.740**	-.064	-.135	-.158
	Sig. (2-tailed)	.304	.043		.507	.008	.002	.399	.230	.006	.819	.645	.589
	N	15	15	15	11	11	12	12	12	12	15	14	14
PFC Connectivity	Pearson Correlation	.433	.240	-.224	1	.484	.136	.049	-.192	.150	.014	.045	.060
	Sig. (2-tailed)	.107	.389	.507		.111	.658	.873	.529	.624	.961	.878	.840
	N	15	15	11	15	12	13	13	13	13	15	14	14
ValleyRH	Pearson Correlation	.484	.307	-.752**	.484	1	.541*	.418	.192	.720**	.116	.013	.140
	Sig. (2-tailed)	.068	.265	.008	.111		.037	.121	.493	.002	.680	.965	.633
	N	15	15	11	12	15	15	15	15	15	15	14	14
ValleyLH	Pearson Correlation	-.050	.043	-.791**	.136	.541*	1	.327	.418	.812**	.331	-.307	-.297
	Sig. (2-tailed)	.855	.875	.002	.658	.037		.216	.107	.000	.211	.265	.282
	N	16	16	12	13	15	16	16	16	16	16	15	15
HillLH	Pearson Correlation	.000	.080	-.268	.049	.418	.327	1	.358	.653**	.038	-.128	-.038

	Sig. (2-tailed)	1.000	.768	.399	.873	.121	.216		.174	.006	.889	.649	.893
	N	16	16	12	13	15	16	16	16	16	16	15	15
HillRH	Pearson Correlation	-.120	.088	-.375	-.192	.192	.418	.358	1	.711**	.329	-.640*	-.525*
	Sig. (2-tailed)	.658	.746	.230	.529	.493	.107	.174		.002	.214	.010	.045
	N	16	16	12	13	15	16	16	16	16	16	15	15
MeanMotor Score	Pearson Correlation	.110	.184	-.740**	.150	.720**	.812**	.653**	.711**	1	.316	-.384	-.272
	Sig. (2-tailed)	.685	.496	.006	.624	.002	.000	.006	.002		.233	.158	.326
	N	16	16	12	13	15	16	16	16	16	16	15	15
BLCort	Pearson Correlation	.243	.407	-.064	.014	.116	.331	.038	.329	.316	1	-.722**	-.764**
	Sig. (2-tailed)	.277	.060	.819	.961	.680	.211	.889	.214	.233		.000	.000
	N	22	22	15	15	15	16	16	16	16	22	20	20
EndCort Change	Pearson Correlation	.084	-.002	-.135	.045	.013	-.307	-.128	-.640*	-.384	-.722**	1	.965**
	Sig. (2-tailed)	.725	.993	.645	.878	.965	.265	.649	.010	.158	.000		.000
	N	20	20	14	14	14	15	15	15	15	20	20	20
MaxCor tChange	Pearson Correlation	.159	.076	-.158	.060	.140	-.297	-.038	-.525*	-.272	-.764**	.965**	1
	Sig. (2-tailed)	.504	.751	.589	.840	.633	.282	.893	.045	.326	.000	.000	
	N	20	20	14	14	14	15	15	15	15	20	20	20

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

REFERENCES

't Hart, B. A., Vogels, J., Bauer, J., Brok, H. P. M., & Blezer, E. (2004). Non-invasive measurement of brain damage in a primate model of multiple sclerosis. *Trends in Molecular Medicine*, *10*(2), 85–91. http://doi.org/10.1016/j.molmed.2003.12.008

Allen, E. A., Erhardt, E. B., Damaraju, E., Gruner, W., Segall, J. M., Silva, R. F., … Calhoun, V. D. (2011). A Baseline for the Multivariate Comparison of Resting-State Networks. *Frontiers in Systems Neuroscience*, *5*. http://doi.org/10.3389/fnsys.2011.00002

Altemus, M., Roca, C., Galliven, E., Romanos, C., & Deuster, P. (2001). Increased vasopressin and adrenocorticotropin responses to stress in the midluteal phase of the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, *86*(6), 2525–2530. http://doi.org/10.1210/jc.86.6.2525

Astur, R. S., Ortiz, M. L., & Sutherland, R. J. (1998). A characterization of performance by men and women in a virtual Morris water task: *Behavioural Brain Research*, *93*(1–2), 185–190. http://doi.org/10.1016/S0166-4328(98)00019-9

Astur, R. S., Purton, A. J., Zaniewski, M. J., Cimadevilla, J., & Markus, E. J. (2016). Human sex differences in solving a virtual navigation problem. *Behavioural Brain Research*, *308*, 236–243. http://doi.org/10.1016/j.bbr.2016.04.037

Bangasser, D. A., & Valentino, R. J. (2014). Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. http://doi.org/10.1016/j.yfrne.2014.03.008

Barel, E. (2016). The role of attentional resources in explaining sex differences in object location memory. *International Journal of Psychology*. http://doi.org/10.1002/ijop.12397

Barth, C., Villringer, A., & Sacher, J. (2015). Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience*, *9*(FEB), 1–20. http://doi.org/10.3389/fnins.2015.00037

Batra, N. A., Seres-Mailo, J., Hanstock, C., Seres, P., Khudabux, J., Bellavance, F., … Le Melledo, J. M. (2008). Proton Magnetic Resonance Spectroscopy Measurement of Brain Glutamate Levels in Premenstrual Dysphoric Disorder. *Biological Psychiatry*, *63*(12), 1178–1184. http://doi.org/10.1016/j.biopsych.2007.10.007

Beckmann, C. F., & Smith, S. M. (2005). Tensorial extensions of independent component analysis for multisubject FMRI analysis. *NeuroImage*, *25*(1), 294–311. http://doi.org/10.1016/j.neuroimage.2004.10.043

Belcher, A. M., Yen, C. C.-C., Notardonato, L., Ross, T. J., Volkow, N. D., Yang, Y., … Tomasi, D. (2016). Functional Connectivity Hubs and Networks in the Awake Marmoset Brain. *Frontiers in Integrative Neuroscience*, *10*(March), 1–9. http://doi.org/10.3389/fnint.2016.00009

Belcher, A. M., Yen, C. C., Stepp, H., Gu, H., Lu, H., Yang, Y., … Stein, E. A. (2013). Large-Scale Brain Networks in the Awake, Truly Resting Marmoset Monkey. *The Journal of Neuroscience*, *33*(42), 16796–16804. http://doi.org/10.1523/JNEUROSCI.3146-13.2013

Beran, M. J., Perdue, B. M., & Smith, J. D. (2014). What are my chances? Closing the gap in uncertainty monitoring between rhesus monkeys (macaca mulatta) and capuchin monkeys (cebus apella). *Journal of Experimental Psychology: Animal Learning and Cognition*, *40*(3), 303–316. http://doi.org/10.1037/xan0000020

Bihel, E., Pro-Sistiaga, P., Letourneur, A., Toutain, J., Saulnier, R., Insausti, R., … Touzani, O. (2010). Permanent or Transient Chronic Ischemic Stroke in the Non-Human Primate: Behavioral, Neuroimaging, Histological, and Immunohistochemical Investigations. *Journal of Cerebral Blood Flow & Metabolism*, *30*(2), 273–285. http://doi.org/10.1038/jcbfm.2009.209

Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*, *20*(11), 4320–4324. http://doi.org/20/11/4320 [pii]

Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M., … Milham, M. P. (2010). Toward discovery science of human brain function. *Proc.Natl.Acad.Sci.U.S.A*, *107*(10), 4734–4739. http://doi.org/0911855107 [pii];10.1073/pnas.0911855107 [doi]

Biswal, B., Yetkin, F., Haughton, V., & Hyde, J. (1995). - Functional connectivity in the motor cortex of resting human brain using. *Magn Reson Med*, *34*(9), 537–541. http://doi.org/10.1002/mrm.1910340409

Bowman, R. E., Beck, K. D., & Luine, V. N. (2003). Chronic stress effects on memory: Sex differences in performance and monoaminergic activity. *Hormones and Behavior*. http://doi.org/10.1016/S0018-506X(02)00022-3

Bowman, R. E., Ferguson, D., & Luine, V. N. (2002). Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. *Neuroscience*, *113*(2), 401–410. http://doi.org/10.1016/S0306-4522(02)00156-2

Braccini, S. N., & Caine, N. G. (2009). Hand preference predicts reactions to novel foods and predators in marmosets (Callithrix geoffroyi). *Journal of Comparative Psychology*, *123*(1), 18–25. http://doi.org/10.1037/a0013089

Bracco, L., Bessi, V., Alari, F., Sforza, A., Barilaro, A., & Marinoni, M. (2011). Cerebral hemodynamic lateralization during memory tasks as assessed by functional transcranial Doppler (fTCD) sonography: Effects of gender and healthy aging. *Cortex*, *47*(6), 750–758. http://doi.org/10.1016/j.cortex.2010.03.007

Brinkman, J., & Kuypers, H. G. J. M. (1973). Cerebral control of contralateral and ipsilateral arm, hand and finger movements in the split-brain rhesus monkey. *Brain*, *96*(4), 653–674. http://doi.org/10.1093/brain/96.4.653

Burish, M. J., Stepniewska, I., & Kaas, J. H. (2008). Microstimulation and Architectonics of Frontoparietal Cortex in Common Marmosets (Callithrix jacchus). *J Comp Neurol*, *507*, 1151–1168. http://doi.org/10.1002/cne

Burman, K. J., Bakola, S., Richardson, K. E., Reser, D. H., & Rosa, M. G. P. (2014). Patterns of cortical input to the primary motor area in the marmoset monkey. *Journal of Comparative Neurology*, *522*(4), 811–843. http://doi.org/10.1002/cne.23447

Calhoun, V. D., & Adali, T. (2006). Unmixing fMRI with independent component analysis. *IEEE Engineering in Medicine and Biology Magazine*, *25*(2), 79–90. http://doi.org/10.1109/MEMB.2006.1607672

Calhoun, V. D., Adali, T., Pearlson, G. D., & Pekar, J. J. (2001). Spatial and temporal independent component analysis of functional MRI data containing a pair of task-related waveforms. *Hum.Brain Mapp.*, *13*(1065–9471), 43–53. http://doi.org/10.1002/hbm.1024

Calhoun, V. D., Adali, T., & Pekar, J. J. (2004). A method for comparing group fMRI data using independent component analysis: Application to visual, motor and visuomotor tasks. *Magnetic Resonance Imaging*, *22*(9), 1181–1191. http://doi.org/10.1016/j.mri.2004.09.004

Camus, S., Ko, W. K. D., Pioli, E., & Bezard, E. (2015). Why bother using non-human primate models of cognitive disorders in translational research? *Neurobiology of Learning and Memory*, *124*, 123–129. http://doi.org/10.1016/j.nlm.2015.06.012

Chai, X. J., & Jacobs, L. F. (2009). Sex differences in directional cue use in a virtual landscape. *Behavioral Neuroscience*, *123*(2), 276–283. http://doi.org/10.1037/a0014722

Chang, L., Ernst, T., Witt, M. D., Ames, N., Gaiefsky, M., & Miller, E. (2002). Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naïve HIV patients. *NeuroImage*, *17*(3), 1638–1648. http://doi.org/10.1006/nimg.2002.1254

Chang, L., Jiang, C. S., & Ernst, T. (2009). Effects of age and sex on brain glutamate and other metabolites. *Magnetic Resonance Imaging*, *27*(1), 142–145. http://doi.org/10.1016/j.mri.2008.06.002

Chaplin, T. A., Yu, H.-H., Soares, J. G. M., Gattass, R., & Rosa, M. G. P. (2013). A Conserved Pattern of Differential Expansion of Cortical Areas in Simian Primates. *Journal of Neuroscience*, *33*(38), 15120–15125. http://doi.org/10.1523/JNEUROSCI.2909-13.2013

Chowdhury, G. M. I., Behar, K. L., Cho, W., Thomas, M. A., Rothman, D. L., & Sanacora, G. (2012). 1H-[13C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biological Psychiatry*, *71*(11), 1022–1025. http://doi.org/10.1016/j.biopsych.2011.11.006

Clarke, H. F., Robbins, T. W., & Roberts, A. C. (2008). Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(43), 10972–10982. http://doi.org/10.1523/JNEUROSCI.1521-08.2008

Collins, P., Wilkinson, L. S., Everitt, B. J., Robbins, T. W., & Roberts, A. C. (2000). The effect of dopamine depletion from the caudate nucleus of the common marmoset (Callithrix jacchus) on tests of prefrontal cognitive function. *Behavioral Neuroscience*, *114*(1), 3–17. http://doi.org/10.1037//0735-7044.114.1.3

Conrad, C. D. (2010). A critical review of chronic stress effects on spatial learning and memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(5), 742–755. http://doi.org/10.1016/j.pnpbp.2009.11.003

Conrad, C. D., Grote, K. A., Hobbs, R. J., & Ferayorni, A. (2003). Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiology of Learning and Memory*, *79*(1), 32–40. http://doi.org/10.1016/S1074-7427(02)00018-7

Cools, R., Clark, L., Owen, A. M., & Robbins, T. W. (2002). Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(11), 4563–4567. http://doi.org/20026435

Cordeiro de Sousa, M., Xavier, N. N., Alves da Silva, H., Souza de Oliveira, M., Yamamoto, M. E., Sousa, M. B. C., & Silva, H. A. da. (2001). Hand preference study in marmosets (*Callithrix jacchus*) using food reaching tests. *Primates*, *42*(1), 57–66. http://doi.org//10.1007/BF02640689

Dias, R., Robbins, T. W., & Roberts, A. C. (1996a). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*. http://doi.org/10.1038/380069a0

Dias, R., Robbins, T. W., & Roberts, a C. (1996b). Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behavioral Neuroscience*, *110*(5), 872–886. http://doi.org/10.1037/0735-7044.110.5.872

Dixson, A. F. (2012). Primate Sexuality: Comparative Studies of the Prosimians, Monkeys, Apes, and Humans, 2nd Edition. In *PRIMATE SEXUALITY: COMPARATIVE STUDIES OF THE PROSIMIANS, MONKEYS, APES, AND HUMANS, 2ND EDITION* (pp. 1–785). http://doi.org/10.1093/acprof:osobl/9780199544646.001.0001

Driscoll, I., Hamilton, D. A., Yeo, R. A., Brooks, W. M., & Sutherland, R. J. (2005). Virtual navigation in humans: The impact of age, sex, and hormones on place learning. *Hormones and Behavior*, *47*(3), 326–335. http://doi.org/10.1016/j.yhbeh.2004.11.013

Duff, S. J., & Hampson, E. (2001). A Sex Difference on a Novel Spatial Working Memory Task in Humans. *Brain and Cognition*, *47*(3), 470–493. http://doi.org/10.1006/brcg.2001.1326

Einstein, G. (2017). Sex and Gender in Health. In *The International Society for Gender Medicine* (pp. 45–55). Elsevier. http://doi.org/10.1016/B978-0-12-811850-4.00006-5

Epting, L. K., & Overman, W. H. (1998). Sex-sensitive tasks in men and women: a search for performance fluctuations across the menstrual cycle. *Behavioral Neuroscience*, *112*(6), 1304–1317. http://doi.org/10.1037/0735-7044.112.6.1304

Eslamboli, A., Baker, H. F., Ridley, R. M., & Annett, L. E. (2003). Sensorimotor deficits in a unilateral intrastriatal 6-OHDA partial lesion model of Parkinson's disease in marmoset monkeys. *Experimental Neurology*, *183*(2), 418–429. http://doi.org/10.1016/S0014-4886(03)00139-0

Evans, K. L., & Hampson, E. (2015). Sex differences on prefrontally-dependent cognitive tasks. *Brain and Cognition*, *93*, 42–53. http://doi.org/10.1016/j.bandc.2014.11.006

Filippi, M., Valsasina, P., Misci, P., Falini, A., Comi, G., & Rocca, M. A. (2013). The organization of intrinsic brain activity differs between genders: A resting-state fMRI study in a large cohort of young healthy subjects. *Human Brain Mapping*, *34*(6), 1330–1343. http://doi.org/10.1002/hbm.21514

Fox, M. D., Corbetta, M., Snyder, A. Z., Vincent, J. L., & Raichle, M. E. (2006). Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proceedings of the National Academy of Sciences*, *103*(26), 10046–10051. http://doi.org/10.1073/pnas.0604187103

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews. Neuroscience*, *8*(9), 700–711. http://doi.org/10.1038/nrn2201

French, J. A., Brewer, K. J., Schaffner, C. M., Schalley, J., Hightower-Merritt, D., Smith, T. E., & Bell, S. M. (1996). Urinary steroid and gonadotropin excretion across the reproductive cycle in female Wied's black tufted-ear marmosets (Callithrix kuhli). *American Journal of Primatology*, *40*(3), 231–245. http://doi.org/10.1002/(SICI)1098-2345(1996)40:3<231::AID-AJP2>3.0.CO;2-Z

Freret, T., Bouet, V., Toutain, J., Saulnier, R., Pro-Sistiaga, P., Bihel, E., … Touzani, O. (2008). Intraluminal thread model of focal stroke in the non-human primate. *Journal of Cerebral Blood Flow and Metabolism*, *28*(4), 786–796. http://doi.org/10.1038/sj.jcbfm.9600575

Friston, K. (2011). Functional and effective connectivity: a review., *1*(1). http://doi.org/10.1089/brain.2011.0008

Frye, C. a, & Walf, A. a. (2004). Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behavioral Neuroscience*, *118*(2), 306–313. http://doi.org/10.1037/0735-7044.118.2.306

Ghahremani, D. G., Monterosso, J., Jentsch, J. D., Bilder, R. M., & Poldrack, R. A. (2010). Neural components underlying behavioral flexibility in human reversal learning. *Cerebral Cortex*, *20*(8), 1843–1852. http://doi.org/10.1093/cercor/bhp247

Gibbs, R. B., Chipman, A. M., Hammond, R., & Nelson, D. (2011). Galanthamine plus estradiol treatment enhances cognitive performance in aged ovariectomized rats. *Hormones and Behavior*, *60*(5), 607–616. http://doi.org/10.1016/j.yhbeh.2011.08.010

Giussani, D. a, Farber, D. M., Jenkins, S. L., Yen, a, Winter, J. a, Tame, J. D., & Nathanielsz, P. W. (2000). Opposing effects of androgen and estrogen on pituitary-adrenal function in nonpregnant primates. *Biology of Reproduction*, *62*(5), 1445–1451. http://doi.org/10.1095/biolreprod62.5.1445

Gordon, D. J., & Rogers, L. J. (2015). Cognitive bias, hand preference and welfare of common marmosets. *Behavioural Brain Research*, *287*, 100–108. http://doi.org/10.1016/j.bbr.2015.03.037

Gujar, S., Maheshwari, S., Bjorkman-Burtscher, I., & Sundgren, P. (2005). Magnetic Resonance Spectroscopy. *Magnetic Resonance Spectroscopy*, *25*(3), 217–226. http://doi.org/10.1016/B978-0-12-401688-0.00014-8

Hädel, S., Wirth, C., Rapp, M., Gallinat, J., & Schubert, F. (2013). Effects of age and sex on the concentrations of glutamate and glutamine in the human brain. *Journal of Magnetic Resonance Imaging*, *38*(6), 1480–1487. http://doi.org/10.1002/jmri.24123

Hall, J. A. Y., & Kimura, D. (1995). Sexual orientation and performance on sexually dimorphic motor tasks. *Archives of Sexual Behavior*, *24*(4), 395–407.

Hampson, E., & Kimura, D. (1988). Reciprocal effects of hormonal fluctuations on human motor and perceptual-spatial skills. *Behavioral Neuroscience*, *102*(3), 456–9. http://doi.org/3395456

Harder, J. A., Aboobaker, A. A., Hodgetts, T. C., & Ridley, R. M. (1998). Learning impairments induced by glutamate blockade using dizocilpine (MK-801) in monkeys. *British Journal of Pharmacology*, *125*(5), 1013–1018. http://doi.org/10.1038/sj.bjp.0702178

Hashimoto, T., Yamazaki, Y., & Iriki, A. (2013). Hand preference depends on posture in common marmosets. *Behavioural Brain Research*, *248*, 1440–150. http://doi.org/10.1016/j.bbr.2013.04.001

Hegarty, M. (2017). Ability and sex differences in spatial thinking: What does the mental rotation test really measure? *Psychonomic Bulletin & Review*. http://doi.org/10.3758/s13423-017-1347-z

Heinzel, S., Metzger, F. G., Ehlis, A.-C., Korell, R., Alboji, A., Haeussinger, F. B., … Fallgatter, A. J. (2013). Aging-related cortical reorganization of verbal fluency processing: a functional near-infrared spectroscopy study. *Neurobiology of Aging*, *34*(2), 439–450. http://doi.org/10.1016/j.neurobiolaging.2012.05.021

Herman, R. a., & Wallen, K. (2007). Cognitive performance in rhesus monkeys varies by sex and prenatal androgen exposure. *Hormones and Behavior*, *51*(4), 496–507. http://doi.org/10.1016/j.yhbeh.2007.01.005

Hikishima, K., Quallo, M. M., Komaki, Y., Yamada, M., Kawai, K., Momoshima, S., … Okano, H. (2011). Population-averaged standard template brain atlas for the common marmoset (Callithrix jacchus). *NeuroImage*, *54*(4), 2741–2749. http://doi.org/10.1016/j.neuroimage.2010.10.061

Hjelmervik, H., Hausmann, M., Osnes, B., Westerhausen, R., & Specht, K. (2014). Resting states are resting traits - An fMRI study of sex differences and menstrual cycle effects in resting state cognitive control networks. *PLoS ONE*, *9*(7). http://doi.org/10.1371/journal.pone.0103492

Honda, A., & Nihei, Y. (2009). Sex differences in object location memory: The female advantage of immediate detection of changes. *Learning and Individual Differences*, *19*(2), 234–237. http://doi.org/10.1016/j.lindif.2008.11.005

Hook, M. A., & Rogers, L. J. (2008). Visuospatial reaching preferences of common marmosets (Callithrix jacchus): An assessment of individual biases across a variety of tasks. *Journal of Comparative Psychology*, *122*(1), 41–51. http://doi.org/10.1037/0735-7036.122.1.41

Horn, D. I., Yu, C., Steiner, J., Buchmann, J., Kaufmann, J., Osoba, A., … Walter, M. (2010). Glutamatergic and resting state functional connectivity correlates of severity in major depression - the role of pregenual anterior cingulate cortex and anterior insula. *Frontiers in Systems Neuroscience*, *4*(July), 1–10. http://doi.org/10.3389/fnsys.2010.00033

Huber, L., Range, F., Voelkl, B., Szucsich, A., Viranyi, Z., & Miklosi, A. (2009). The evolution of imitation: what do the capacities of non-human animals tell us about the mechanisms of imitation? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1528), 2299–2309. http://doi.org/10.1098/rstb.2009.0060

Hutchison, R. M., Leung, L. S., Mirsattari, S. M., Gati, J. S., Menon, R. S., & Everling, S. (2011). Resting-state networks in the macaque at 7T. *NeuroImage*, *56*(3), 1546–1555. http://doi.org/10.1016/j.neuroimage.2011.02.063

Ingalhalikar, M., Smith, A., Parker, D., Satterthwaite, T. D., Elliott, M. a, Ruparel, K., … Verma, R. (2014). Sex differences in the structural connectome of the human brain. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(2), 823–8. http://doi.org/10.1073/pnas.1316909110

Izquierdo, A. (2004). Bilateral Orbital Prefrontal Cortex Lesions in Rhesus Monkeys Disrupt Choices Guided by Both Reward Value and Reward Contingency. *Journal of Neuroscience*, *24*(34), 7540–7548. http://doi.org/10.1523/JNEUROSCI.1921-04.2004

Izquierdo, A., Brigman, J. L., Radke, A. K., Rudebeck, P. H., & Holmes, A. (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience*. http://doi.org/10.1016/j.neuroscience.2016.03.021

Jacobs, E., & D'Esposito, M. (2011). Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(14), 5286–5293. http://doi.org/10.1523/JNEUROSCI.6394-10.2011

Jennings, P. J., Janowsky, J. S., & Orwoll, E. (1998). Estrogen and sequential movement. *Behavioral Neuroscience*, *112*(1), 154–159.

Johnson-Frey, S. H. (2004). The neural bases of complex tool use in humans. *Trends in Cognitive Sciences*, *8*(2), 71–78. http://doi.org/10.1016/j.tics.2003.12.002

Johnson, E. O., Kamilaris, T. C., Carter, C. S., Calogero, A. E., Gold, P. W., & Chrousos, G. P. (1996). The biobehavioral consequences of psychogenic stress in a small, social primate (Callithrix jacchus jacchus). *Biological Psychiatry*, *40*(5), 317–337. http://doi.org/10.1016/0006-3223(95)00397-5

Jung, R. E., Gasparovic, C., Chavez, R. S., Caprihan, A., Barrow, R., & Yeo, R. A. (2009). Imaging intelligence with proton magnetic resonance spectroscopy. *Intelligence*, *37*(2), 192–198. http://doi.org/10.1016/j.intell.2008.10.009

Jung, R. E., Haier, R. J., Yeo, R. A., Rowland, L. M., Petropoulos, H., Levine, A. S., … Brooks, W. M. (2005). Sex differences in N-acetylaspartate correlates of general intelligence: An 1H-MRS study of normal human brain. *NeuroImage*, *26*(3), 965–972. http://doi.org/10.1016/j.neuroimage.2005.02.039

Kangas, B. D., & Bergman, J. (2017). Touchscreen technology in the study of cognition-related behavior. *Behavioral Pharmacology*. http://doi.org/10.1097/FBP.0000000000000356

Kaufman, M. J., Janes, A. C., Hudson, J. I., Brennan, B. P., Kanayama, G., Kerrigan, A. R., … Pope, H. G. (2015). Brain and cognition abnormalities in long-term anabolic-androgenic steroid users. *Drug and Alcohol Dependence*, *152*(152), 47–56. http://doi.org/10.1016/j.drugalcdep.2015.04.023

Korol, D. L., & Pisani, S. L. (2015). Estrogens and cognition: Friends or foes? An evaluation of the opposing effects of estrogens on learning and memory. *Hormones and Behavior*, *74*, 105–115. http://doi.org/10.1016/j.yhbeh.2015.06.017

LaClair, M., & Lacreuse, A. (2016). Reversal learning in gonadectomized marmosets with and without hormone replacement: are males more sensitive to punishment? *Animal Cognition*, *19*(3), 619–630. http://doi.org/10.1007/s10071-016-0966-5

Lacreuse, A., Chang, J., Metevier, C. M., Laclair, M., Meyer, J. S., & Ferris, C. M. (2014). Oestradiol modulation of cognition in adult female marmosets (Callithrix jacchus). *Journal of Neuroendocrinology*, *26*(5), 296–309.

Lacreuse, A., & Herndon, J. G. (2009). Nonhuman Primate Models of Cognitive Aging. In *Animal Models of Human Cognitive Aging* (pp. 29–58). http://doi.org/10.1007/978-1-59745-422-3

Lacreuse, A., Kim, C. B., Rosene, D. L., Killiany, R. J., Moss, M. B., Moore, T. L., … Herndon, J. G. (2005). Sex, age, and training modulate spatial memory in the rhesus monkey (Macaca mulatta). *Behavioral Neuroscience*, *119*(1), 118–126. http://doi.org/10.1037/0735-7044.119.1.118

Lai, Z. C., Moss, M. B., Killiany, R. J., Rosene, D. L., & Herndon, J. G. (1995). Executive system dysfunction in the aged monkey: Spatial and object reversal learning. *Neurobiology of Aging*, *16*(6), 947–954. http://doi.org/10.1016/0197-4580(95)02014-4

Le Gal, R., Bernaudin, M., Toutain, J., & Touzani, O. (2017). Assessment of behavioural deficits following ischaemic stroke in the marmoset. *Behavioural Brain Research*, (April), 0–1. http://doi.org/10.1016/j.bbr.2017.07.042

Lejbak, L., Vrbancic, M., & Crossley, M. (2009). The female advantage in object location memory is robust to verbalizability and mode of presentation of test stimuli. *Brain and Cognition*, *69*(1), 148–153. http://doi.org/10.1016/j.bandc.2008.06.006

Levine, S., Vasilyeva, M., Lourenco, S. F., Newcombe, N. S., Huttenlocher, J., Report, R., & Report, R. (2005). Socioeconomic Status Modfies the Sex Difference in Spatial Skill. *Psychological Science*, *16*(11), 841–845. Retrieved from http://www.jstor.org/stable/40064325

Lippa, R. A., Collaer, M. L., & Peters, M. (2010). Sex differences in mental rotation and line angle judgments are positively associated with gender equality and economic development across 53 nations. *Archives of Sexual Behavior*, *39*(4), 990–997. http://doi.org/10.1007/s10508-008-9460-8

Liu, J. J. W., Ein, N., Peck, K., Huang, V., Pruessner, J. C., & Vickers, K. (2017). Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis. *Psychoneuroendocrinology*, *82*(October 2016), 26–37. http://doi.org/10.1016/j.psyneuen.2017.04.007

Luine, V., Gomez, J., Beck, K., & Bowman, R. (2017). Sex differences in chronic stress effects on cognition in rodents. *Pharmacology Biochemistry and Behavior*, *152*, 13–19. http://doi.org/10.1016/j.pbb.2016.08.005

Lupien, S. J., & Lepage, M. (2001). Stress, memory, and the hippocampus: Can't live with it, can't live without it. *Behavioural Brain Research*, *127*(1–2), 137–158. http://doi.org/10.1016/S0166-4328(01)00361-8

MacDonald, S., Pang, J., & Gibeault, S. (1994). Marmoset (Callithrix jacchus jacchus) Spatial Memory in a Foraging Task. *Journal of Comparative Psychology*, *108*(4), 328–334. Retrieved from https://www.researchgate.net/profile/Suzanne_Macdonald/publication/15386400_Marmoset_Callithrix_jacchus_jacchus_spatial_memory_in_a_foraging_task_Winstay_versus_winshift_strategies/links/00b49533cc3133b574000000/Marmoset-Callithrix-jacchus-jacchus-spatial

Machado, C. J., & Bachevalier, J. (2007). The effects of selective amygdala, orbital frontal cortex or hippocampal formation lesions on reward assessment in nonhuman primates. *European Journal of Neuroscience*, *25*(9), 2885–2904. http://doi.org/10.1111/j.1460-9568.2007.05525.x

Magistretti, P. J. (2011). Neuron-glia metabolic coupling and plasticity. *Experimental Physiology*, *96*(4), 407–410. http://doi.org/10.1113/expphysiol.2010.053157

Maki, P. M., Rich, J. B., & Shayna Rosenbaum, R. (2002). Implicit memory varies across the menstrual cycle: Estrogen effects in young women. *Neuropsychologia*, *40*(5), 518–529. http://doi.org/10.1016/S0028-3932(01)00126-9

Mantini, D., Corbetta, M., Romani, G. L., Orban, G. A., & Vanduffel, W. (2013). Evolutionarily Novel Functional Networks in the Human Brain? *Journal of Neuroscience*, *33*(8), 3259–3275. http://doi.org/10.1523/JNEUROSCI.4392-12.2013

Marshall, J. W. B., Ridley, R. M., Baker, H. F., Hall, L. D., Carpenter, T. A., & Wood, N. I. (2003). Serial MRI, functional recovery, and long-term infarct maturation in a non-human primate model of stroke. *Brain Research Bulletin*, *61*(6), 577–585. http://doi.org/10.1016/S0361-9230(03)00214-4

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research*, *146*(1–2), 97–103. http://doi.org/10.1016/j.bbr.2003.09.019

McCarrey, A. C., An, Y., Kitner-Triolo, M. H., Ferrucci, L., & Resnick, S. M. (2016). Sex differences in cognitive trajectories in clinically normal older adults. *Psychology and Aging*, *31*(2), 166–175. http://doi.org/10.1037/pag0000070

McGregor, H. R., & Gribble, P. L. (2017). Functional connectivity between somatosensory and motor brain areas predicts individual differences in motor learning by observing. *Journal of Neurophysiology*, *118*(2), 1235–1243. http://doi.org/10.1152/jn.00275.2017

McKeown, M. J., Makeig, S., Brown, G. G., Jung, T. P., Kindermann, S. S., Bell, A. J., & Sejnowski, T. J. (1998). Analysis of fMRI data by blind separation into independent spatial components. *Human Brain Mapping*, *6*(3), 160–188. http://doi.org/10.1002/(SICI)1097-0193(1998)6:3<160::AID-HBM5>3.0.CO;2-1

McLaughlin, K. J., Baran, S. E., Wright, R. L., & Conrad, C. D. (2005). Chronic stress enhances spatial memory in ovariectomized female rats despite CA3 dendritic retraction: Possible involvement of CA1 neurons. *Neuroscience*, *135*(4), 1045–1054. http://doi.org/10.1016/j.neuroscience.2005.06.083

Merritt, K., Egerton, A., Kempton, M. J., Taylor, M. J., & McGuire, P. K. (2016). Nature of glutamate alterations in schizophrenia a meta-analysis of proton magnetic resonance spectroscopy studies. *JAMA Psychiatry*, *73*(7), 665–674. http://doi.org/10.1001/jamapsychiatry.2016.0442

Meyer, J. S., Brevard, M. E., Piper, B. J., Ali, S. F., & Ferris, C. F. (2006). Neural effects of MDMA as determined by functional magnetic resonance imaging and magnetic resonance spectroscopy in awake marmoset monkeys. *Annals of the New York Academy of Sciences*, *1074*, 365–376. http://doi.org/10.1196/annals.1369.036

Miles, R. C. (1957). Delayed-response learning in the marmoset and the macaque. *Journal of Comparative and Physiological Psychology*, *50*(4), 352–355. http://doi.org/10.1037/h0040257

Miller, D. I., & Halpern, D. F. (2014). The new science of cognitive sex differences. *Trends in Cognitive Sciences*, *18*(1), 37–45. http://doi.org/10.1016/j.tics.2013.10.011

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(8), 2921–2927. http://doi.org/10.1016/0091-3057(93)90217-H

Morgan, M. a, & Pfaff, D. W. (2001). Effects of estrogen on activity and fear-related behaviors in mice. *Hormones and Behavior*, *40*(4), 472–482. http://doi.org/10.1006/hbeh.2001.1716

Mueller, S. C., Wierckx, K., Jackson, K., & T'Sjoen, G. (2016). Circulating androgens correlate with resting-state MRI in transgender men. *Psychoneuroendocrinology*, *73*, 91–98. http://doi.org/10.1016/j.psyneuen.2016.07.212

Munro, C. A., Winicki, J. M., Schretlen, D. J., Gower, E. W., Turano, K. A., Muñoz, B., … West, S. K. (2012). Sex Differences in Cognition in Healthy Elderly Individuals. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn*, *19*(6), 759–768. http://doi.org/10.1080/13825585.2012.690366.Sex

Murre, J. M. J., Janssen, S. M. J., Rouw, R., & Meeter, M. (2013). The rise and fall of immediate and delayed memory for verbal and visuospatial information from late childhood to late adulthood. *Acta Psychologica*, *142*(1), 96–107. http://doi.org/10.1016/j.actpsy.2012.10.005

Nagahama, Y., Okada, T., Katsumi, Y., Hayashi, T., Yamauchi, H., Oyanagi, C., … Shibasaki, H. (2001). Dissociable mechanisms of attentional control within the human prefrontal cortex. *Cerebral Cortex*, *11*(1), 85–92. http://doi.org/10.1093/cercor/11.1.85

Nikolova, S., Stark, S. M., & Stark, C. E. L. (2017). 3T hippocampal glutamate-glutamine complex reflects verbal memory decline in aging. *Neurobiology of Aging*, *54*, 103–111. http://doi.org/10.1016/j.neurobiolaging.2017.01.026

Nishijima, K., Saitoh, R., Tanaka, S., Ohsato-Suzuki, M., Ohno, T., & Kitajima, S. (2012). Life span of common marmoset (Callithrix jacchus) at CLEA Japan breeding colony. *Biogerontology*, *13*(4), 439–443. http://doi.org/10.1007/s10522-012-9388-1

Overman, W. H. (2004). Sex differences in early childhood, adolescence, and adulthood on cognitive tasks that rely on orbital prefrontal cortex. *Brain and Cognition*, *55*(1), 134–147. http://doi.org/10.1016/S0278-2626(03)00279-3

Owen, A. M., Roberts, A. C., Polkey, C. E., Sahakian, B. J., & Robbins, T. W. (1991). Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*, *29*(10), 993–1006. http://doi.org/10.1016/0028-3932(91)90063-E

Oyola, M. G., & Handa, R. J. (2017). Hypothalamic–pituitary–adrenal and hypothalamic–pituitary– gonadal axes: sex differences in regulation of stress responsivity HHS Public Access. *Stress*, *20*(5), 476–494. http://doi.org/10.1080/10253890.2017.1369523

Patel, T., Blyth, J. C., Griffiths, G., Kelly, D., & Talcott, J. B. (2014). Moderate relationships between NAA and cognitive ability in healthy adults: implications for cognitive spectroscopy. *Frontiers in Human Neuroscience*, *8*(February), 39. http://doi.org/10.3389/fnhum.2014.00039

Paxinos, G., Watson, C., Petrides, M., Rosa, M., & Tokuno, H. (2012). The marmoset brain in stereotaxic coordinates. Retrieved from https://espace.curtin.edu.au/handle/20.500.11937/40725

Pearce, P. C., Crofts, H. S., Muggleton, N. G., & Scott, E. A. M. (1998). Concurrent monitoring of EEG and performance in the common marmoset: A methodological approach. *Physiology and Behavior*, *63*(4), 591–599. http://doi.org/10.1016/S0031-9384(97)00494-0

Persson, J., Herlitz, A., Engman, J., Morell, A., Sjölie, D., Wikström, J., & Söderlund, H. (2013). Remembering our origin: Gender differences in spatial memory are reflected in gender differences in hippocampal lateralization. *Behavioural Brain Research*, *256*, 219–228. http://doi.org/10.1016/j.bbr.2013.07.050

Petersen, N., Kilpatrick, L. A., Goharzad, A., & Cahill, L. (2014). Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. *NeuroImage*, *90*, 24–32. http://doi.org/10.1016/j.neuroimage.2013.12.016

Phillips, K. A., Ross, C. N., Spross, J., Cheng, C. J., Izquierdo, A., Biju, K. C., … Tardif, S. D. (2017). Behavioral phenotypes associated with MPTP induction of partial lesions in common marmosets (Callithrix jacchus). *Behavioural Brain Research*, *325*, 51–62. http://doi.org/10.1016/j.bbr.2017.02.010

Piddington, T., & Rogers, L. J. (2009). Hand and paw preferences in relation to the lateralized brain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1519), 943–954. http://doi.org/10.1098/rstb.2008.0225

Preuss, T. M. (2012). Human brain evolution: from gene discovery to phenotype discovery. *Proceedings of the National Academy of Sciences of the United States of America*, *109 Suppl*(SUPPL.1), 10709–16. http://doi.org/10.1073/pnas.1201894109

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, *30*(6), 672–670.

Raichle, M. E. (2015). The Brain's Default Mode Network. *Annual Review of Neuroscience*, *38*(1), 433–447. http://doi.org/10.1146/annurev-neuro-071013-014030

Ramadan, S., Lin, A., & Stanwell, P. (2013). Glutamate and glutamine: A review of in vivo MRS in the human brain. *NMR in Biomedicine*. http://doi.org/10.1002/nbm.3045

Ridley, R. M., Haystead, T. A. J., & Baker, H. F. (1981). An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol. *Pharmacology, Biochemistry and Behavior*, *14*(3), 345–351. http://doi.org/10.1016/0091-3057(81)90401-9

Riedel, G., Platt, B., & Micheau, J. (2003). Glutamate receptor function in learning and memory. *Behavioural Brain Research*, *140*(1–2), 1–47. http://doi.org/10.1016/S0166-4328(02)00272-3

Rilling, J. K. (2014). Comparative primate neuroimaging: Insights into human brain evolution. *Trends in Cognitive Sciences*. http://doi.org/10.1016/j.tics.2013.09.013

Robbins, T. W., James, M., Owen, A. M., Sahakian, B. J., McInnes, L., & Rabbitt, P. (1994). Cambridge Neuropsychological Test Automated Battery (CANTAB): A Factor Analytic Study of a Large Sample of Normal Elderly Volunteers. *Dementia and Geriatric Cognitive Disorders*, *5*(5), 266–281. http://doi.org/10.1159/000106735

Roberts, A. C. (1996). Comparison of cognitive function in human and non-human primates. *Cognitive Brain Research*, *3*(3–4), 319–327. http://doi.org/10.1016/0926-6410(96)00017-1

Roberts, a C., Robbins, T. W., & Everitt, B. J. (1988). The effects of intradimensional and extradimensional shifts on visual discrimination learning in humans and non-human primates. *The Quarterly Journal of Experimental Psychology. B, Comparative and Physiological Psychology*, *40*(4), 321–341. http://doi.org/10.1080/14640748808402328

Roser, W., Hagberg, G., Mader, I., Brunnschweiler, H., Radue, E. W., Seelig, J., & Kappos, L. (1995). Proton MRS of Gadolinium-enhancing MS Plaques and Metabolic Changes in Normal-Appearing White Matter. *Magnetic Resonance in Medicine*, *33*(6), 811–817. http://doi.org/10.1002/mrm.1910330611

Ross, A. J., & Sachdev, P. S. (2004). Magnetic resonance spectroscopy in cognitive research. *Brain Research Reviews*, *44*(2–3), 83–102. http://doi.org/10.1016/j.brainresrev.2003.11.001

Rutgers, D. R., Klijn, C. J., Kappelle, L. J., & van der Grond, J. (2000). Cerebral metabolic changes in patients with a symptomatic occlusion of the internal carotid artery: a longitudinal 1H magnetic resonance spectroscopy study. *J Magn Reson Imaging*, *11*(3), 279–286. http://doi.org/10.1002/(SICI)1522-2586(200003)11:3<279::AID-JMRI6>3.0.CO;2-0

Sala-Llonch, R., Peña-Gómez, C., Arenaza-Urquijo, E. M., Vidal-Piñeiro, D., Bargalló, N., Junqué, C., & Bartrés-Faz, D. (2012). Brain connectivity during resting state and subsequent working memory task predicts behavioural performance. *Cortex*, *48*(9), 1187–1196. http://doi.org/10.1016/j.cortex.2011.07.006

Salomons, A. R., Arndt, S. S., & Ohl, F. (2012). Impact of anxiety profiles on cognitive performance in BALB/c and 129P2 mice. *Cognitive, Affective and Behavioral Neuroscience*, *12*(4), 794–803. http://doi.org/10.3758/s13415-012-0109-7

Sandstrom, N. J., Kaufman, J., & A. Huettel, S. (1998). Males and females use different distal cues in a virtual environment navigation task. *Cognitive Brain Research*, *6*(4), 351–360. http://doi.org/10.1016/S0926-6410(98)00002-0

Schwabe, L., Joëls, M., Roozendaal, B., Wolf, O. T., & Oitzl, M. S. (2012). Stress effects on memory: An update and integration. *Neuroscience and Biobehavioral Reviews*, *36*(7), 1740–1749. http://doi.org/10.1016/j.neubiorev.2011.07.002

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., … Greicius, M. D. (2007). Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *Journal of Neuroscience*, *27*(9), 2349–2356. http://doi.org/10.1523/JNEUROSCI.5587-06.2007

Sereno, M. I., & Tootell, R. B. H. (2005). From monkeys to humans: What do we now know about brain homologies? *Current Opinion in Neurobiology*, *15*(2), 135–144. http://doi.org/10.1016/j.conb.2005.03.014

Siegel, J. A., Young, L. A., Neiss, M. B., Samuels, M. H., Roselli, C. E., & Janowsky, J. S. (2008). Estrogen, testosterone, and sequential movement in men. *Behavioral Neuroscience*, *122*(5), 955–962. http://doi.org/10.1037/a0013045

Silva, A. C., Liu, J. V, Hirano, Y., Leoni, R. F., Merkle, H., Mackel, J. B., … Stefanovic, B. (2011). Longitudinal Functional Magnetic Resonance Imaging in Animal Models (pp. 281–302). http://doi.org/10.1007/978-1-61737-992-5_14

Simmons, M. L., Frondoza, C. G., & Coyle, J. T. (1991). Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience*, *45*(1), 37–45. http://doi.org/10.1016/0306-4522(91)90101-S

Smith, S. M. (2002). Fast robust automated brain extraction. *Human Brain Mapping*, *17*(3), 143–155. http://doi.org/10.1002/hbm.10062

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., … Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. In *NeuroImage* (Vol. 23, pp. 208–219). http://doi.org/10.1016/j.neuroimage.2004.07.051

Somogyi, P., Tamás, G., Lujan, R., & Buhl, E. H. (1998). Salient features of synaptic organisation in the cerebral cortex. In *Brain Research Reviews* (Vol. 26, pp. 113–135). http://doi.org/10.1016/S0165-0173(97)00061-1

Spinelli, S., Pennanen, L., Dettling, A. C., Feldon, J., Higgins, G. A., & Pryce, C. R. (2004). Performance of the marmoset monkey on computerized tasks of attention and working memory. *Cognitive Brain Research*, *19*(2), 123–137. http://doi.org/10.1016/j.cogbrainres.2003.11.007

Stavisky, R. C., Watson, S. L., Anthony, M. S., Manuck, S. B., Adams, M. R., & Kaplan, J. R. (2003). Influence of estradiol on cortisol secretion in ovariectomized cynomolgus macaques (Macaca fascicularis). *American Journal of Primatology*, *60*(1), 17–22. http://doi.org/10.1002/ajp.10076

Steele, C. M., Spencer, S. J., & Aronson, J. (2002). Contending with group image: The psychology of stereotype and social identity threat (pp. 379–440). http://doi.org/10.1016/S0065-2601(02)80009-0

Strasser, A., & Burkart, J. M. (2012). Can we measure brain efficiency? An empirical test with common marmosets (Callithrix jacchus). *Brain, Behavior and Evolution*, *80*(1), 26–40. http://doi.org/10.1159/000338014

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: Social rejection versus achievement stress. *Biological Psychiatry*, *52*(4), 318–327. http://doi.org/10.1016/S0006-3223(02)01333-1

Stroud, L. R., Tanofsky-Kraff, M., Wilfley, D. E., & Salovey, P. (2000). The Yale Interpersonal Stressor (YIPS): Affective, physiological, and behavioral responses to a novel interpersonal rejection paradigm. *Annals of Behavioral Medicine*, *22*(3), 204–213. http://doi.org/10.1007/BF02895115

Takemoto, A., Miwa, M., Koba, R., Yamaguchi, C., Suzuki, H., & Nakamura, K. (2015). Individual variability in visual discrimination and reversal learning performance in common marmosets. *Neuroscience Research*, *93*, 136–143. http://doi.org/10.1016/j.neures.2014.10.001

Tardif, S. D., & Bales, K. L. (2004). Relations among birth condition, maternal condition, and postnatal growth in captive common marmoset monkeys (Callithrix jacchus). *American Journal of Primatology*, *62*(2), 83–94. http://doi.org/10.1002/ajp.20009

Taylor, R., Neufeld, R. W. J., Schaefer, B., Densmore, M., Rajakumar, N., Osuch, E. A., … Théberge, J. (2015). Functional magnetic resonance spectroscopy of glutamate in schizophrenia and major depressive disorder: anterior cingulate activity during a color-word Stroop task. *Npj Schizophrenia*, *1*(1), 15028. http://doi.org/10.1038/npjschz.2015.28

Taylor, R., Schaefer, B., Densmore, M., Neufeld, R. W. J., Rajakumar, N., Williamson, P. C., & Th??berge, J. (2015). Increased glutamate levels observed upon functional activation in the anterior cingulate cortex using the Stroop Task and functional spectroscopy. *NeuroReport*, *26*(3), 107–112. http://doi.org/10.1097/WNR.0000000000000309

Tian, L., Wang, J., Yan, C., & He, Y. (2011). Hemisphere- and gender-related differences in small-world brain networks: A resting-state functional MRI study. *NeuroImage*, *54*(1), 191–202. http://doi.org/10.1016/j.neuroimage.2010.07.066

Van Den Heuvel, M. P., Mandl, R. C. W., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Human Brain Mapping*, *30*(10), 3127–3141. http://doi.org/10.1002/hbm.20737

van Vlieta, S. A. M., Blezer, E. L. A., Jongsma, M. J., Vanwersch, R. A. P., Olivier, B., & Philippens, I. H. C. H. M. (2008a). Exploring the neuroprotective effects of modafinil in a marmoset Parkinson model with immunohistochemistry, magnetic resonance imaging and spectroscopy. *Brain Research*, *1189*(1), 219–228. http://doi.org/10.1016/j.brainres.2007.10.059

van Vlieta, S. A. M., Blezer, E. L. A., Jongsma, M. J., Vanwersch, R. A. P., Olivier, B., & Philippens, I. H. C. H. M. (2008b). Exploring the neuroprotective effects of modafinil in a marmoset Parkinson model with immunohistochemistry, magnetic resonance imaging and spectroscopy. *Brain Research*, *1189*(1), 219–228. http://doi.org/10.1016/j.brainres.2007.10.059

Visu-Petra, L., Miclea, M., & Visu-Petra, G. (2013). Individual differences in anxiety and executive functioning: A multidimensional view. *International Journal of Psychology*, *48*(4), 649–659. http://doi.org/10.1080/00207594.2012.656132

Voelkl, B., & Huber, L. (2000). True imitation in marmosets. *Animal Behaviour*, *60*(2), 195–202. http://doi.org/10.1006/anbe.2000.1457

Voyer, D., Postma, A., Brake, B., & Imperato-McGinley, J. (2007). Gender differences in object location memory: A meta-analysis. *Psychonomic Bulletin & Review*, *14*(1), 23–38. http://doi.org/10.3758/BF03194024

Voyer, D., Voyer, S., Bryden, M. P., Bigelow, A., Brooks, G. P., Bulman-Fleming, B., … Johnson, R. W. (1995). Magnitude of Sex Differences in Spatial Abilities: A Meta-Analysis and Consideration of Critical Variables. *Psychological Bulletin*, *117*(2), 250–270. http://doi.org/10.1037/0033-2909.117.2.250

Wagner, G., Gussew, A., Köhler, S., de la Cruz, F., Smesny, S., Reichenbach, J. R., & Bär, K. J. (2016). Resting state functional connectivity of the hippocampus along the anterior-posterior axis and its association with glutamatergic metabolism. *Cortex*, *81*, 104–117. http://doi.org/10.1016/j.cortex.2016.03.022

Wang, V. C., Neese, S. L., Korol, D. L., & Schantz, S. L. (2009). Chronic estradiol replacement impairs performance on an operant delayed spatial alternation task in young, middle-aged, and old rats. *Hormones and Behavior*, *56*(4), 382–390. http://doi.org/10.1016/j.yhbeh.2009.07.005

Weed, M. R., Taffe, M. A., Polis, I., Roberts, A. C., Robbins, T. W., Koob, G. F., … Gold, L. H. (1999). Performance norms for a rhesus monkey neuropsychological testing battery: Acquisition and long-term performance. *Cognitive Brain Research*, *8*(3), 185–201. http://doi.org/10.1016/S0926-6410(99)00020-8

Weiss, E. M., Ragland, J. D., Brensinger, C. M., Bilker, W. B., Deisenhammer, E. A., & Delazer, M. (2006). Sex differences in clustering and switching in verbal fluency tasks. *Journal of the International Neuropsychological Society*, *12*(4), 502–509. http://doi.org/10.1017/S1355617706060656

Weissman-Fogel, I., Moayedi, M., Taylor, K. S., Pope, G., & Davis, K. D. (2010). Cognitive and default-mode resting state networks: Do male and female brains "rest" differently? *Human Brain Mapping*, *31*(11), 1713–1726. http://doi.org/10.1002/hbm.20968

Woodcock, E. A., Anand, C., Khatib, D., Diwadkar, V. A., & Stanley, J. A. (2018). Working Memory Modulates Glutamate Levels in the Dorsolateral Prefrontal Cortex during 1H fMRS. *Frontiers in Psychiatry*, *9*(March), 66. http://doi.org/10.3389/fpsyt.2018.00066

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., … Smith, S. M. (2008). Bayesian analysis of neuroimaging data in FSL ☆. *NeuroImage*, *45*, S173–S186. http://doi.org/10.1016/j.neuroimage.2008.10.055

Yamazaki, Y., Echigo, C., Saiki, M., Inada, M., Watanabe, S., & Iriki, A. (2011). Tool-use learning by common marmosets (Callithrix jacchus). *Experimental Brain Research*, *213*(1), 63–71. http://doi.org/10.1007/s00221-011-2778-9

Yamazaki, Y., Saiki, M., Inada, M., Watanabe, S., & Iriki, A. (2016). Sustained performance by common marmosets in a delayed matching to position task with variable stimulus presentations. *Behavioural Brain Research*, *297*, 277–284. http://doi.org/10.1016/j.bbr.2015.10.025

Yang, G. J., Murray, J. D., Repovs, G., Cole, M. W., Savic, A., Glasser, M. F., … Anticevic, A. (2014). Altered global brain signal in schizophrenia. *Proceedings of the National Academy of Sciences*, *111*(20), 7438–7443. http://doi.org/10.1073/pnas.1405289111

Zaletel, I., Filipvic, D., & Puskas, N. (2016). Chronic stress, hippocampus and parvalbumin-positive interneurons: what do we know so far? *Rev. Neurosci.*, *27*(4), 397–409.

Zhang, B., Li, S., Zhuo, C., Li, M., Safron, A., Genz, A., … Walter, M. (2017). Altered task-specific deactivation in the default mode network depends on valence in patients with major depressive disorder. *Journal of Affective Disorders*, *207*(August 2016), 377–383. http://doi.org/10.1016/j.jad.2016.08.042

Zhao, Q., Li, H., Yu, X., Huang, F., Wang, Y., Liu, L., … Wang, Y. (2017). Abnormal resting-state functional connectivity of insular subregions and disrupted correlation with working memory in adults with attention deficit/hyperactivity disorder. *Frontiers in Psychiatry*, *8*(OCT), 1–10. http://doi.org/10.3389/fpsyt.2017.00200

Zou, Q., Ross, T. J., Gu, H., Geng, X., Zuo, X. N., Hong, L. E., … Yang, Y. (2013). Intrinsic resting-state activity predicts working memory brain activation and behavioral performance. *Human Brain Mapping*, *34*(12), 3204–3215. http://doi.org/10.1002/hbm.22136